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IN-UTERO AND EARLY LIFE ORIGINS OF ADIPOSITY IN INFANTS BORN TO OBESE MOTHERS

NASHITA PATEL

A thesis submitted to King's College London for the degree of
Doctor of Philosophy
Division of Women's Health
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Abbreviations

ALSPAC- Avon Longitudinal Study of Parents and Children

AOR- Adjusted odds ratio

ARR- Adjusted risk ratio

BEBQ- Baby eating behaviour questionnaire

BMI- Body mass index

CDM- Covariate dependent missing

CRP- C Reactive Protein

DAG- Direct acyclic graphs

DEXA- Dual Energy X-ray absorptiometry

DOHAD- Developmental origins of health and disease

FDR- False discovery rate

FFQ- Food frequency questionnaire

FMI- Fat mass index

FTO- Fat mass and obesity associated gene

HDLc- High density lipoprotein cholesterol

GDM- Gestational diabetes

GWAS- Genome wide association studies

GWG- Gestational weight gain

HAPO- Hyperglycaemia and Adverse Pregnancy Outcome

HPA- Hypothalamic pituitary axis

IADPSG- International Association of Diabetes and Pregnancy Study Group

IBQ-R- Revised infant behaviour questionnaire

IGF- Insulin growth factor

IL-6- Interleukin 6

IOM- Institute of Medicine

IPAQ- International Physical Activity Questionnaire

IQR- Interquartile range

IUGR- Intrauterine growth restriction

LDLc- Low density lipoprotein cholesterol

LGA- Large for gestational age

LIMIT- Limiting weight gain in overweight and obese women during pregnancy to improve health outcomes.

LPC- Lysophosphatidylcholine

MAR- Missing at random

MCAR- Missing completely at random

MET- Metabolic equivalent of task

MNAR- Missing not at random

MR- Mendelian Randomisation

MS- Mass spectroscopy

NMR- Nuclear magnetic spectroscopy

OGTT- Oral glucose tolerance test

OR- Odds ratio

SD- Standard deviation

SFTs- Skinfold thicknesses

SGA- Small for gestational age

SNP- Single nucleotide polymorphisms

SSFTs- Sum of skinfold thicknesses

TNF- α - Tumour Necrosis Factor

UK- United Kingdom

UPBEAT- UK Pregnancies Better Eating and Activity Trial

USA- United States of America

Statement of contribution

All aspects of the UK Pregnancies Better Eating and Activity Study were proposed and devised by the principal investigator and co investigators (full list of contributors can be found on the study website; www.medscinet.net/upbeat/default.aspx).

This thesis reports the results of follow-up of offspring born to obese pregnant women from birth to 6 months of age. The author formulated and undertook the statistical analysis for reporting the primary outcomes from the main study, under the supervision of Professor Lucilla Poston (primary supervisor) and Dr Dharmintra Pasupathy (secondary supervisor). The author was responsible for data management, statistical analysis and interpretation of findings during the course of the PhD. Assessment of anthropometry were devised by Professor Lucilla Poston and Professor Keith Godfrey. Additional epidemiology and statistical supervision was provided by Mr Paul Seed, Professor Keith Godfrey and Professor Debbie Lawlor. Recruitment and data collection was carried out by research midwives at each of the eight participating centres under the supervision of Dr Annette Briley and Claire Singh. All biochemical assays were undertaken by collaborators of the UK Pregnancies Better Eating and Activity Study, at accredited laboratories. The cord blood metabolomic analysis was undertaken under the supervision of Dr Christain Hellmuth at Ludwig Maximilians University, Munich.

Abstract

Experimental animal models and observational studies suggest that maternal obesity in pregnancy influences the development of obesity in early infancy. As the trajectory of later adiposity is thought to be determined in early life; an understanding of potential contributing factors and associative mechanisms are essential to inform targeted interventions.

The analyses reported in this thesis have sought to address these associations in mothers and their offspring from the UK Pregnancies Better Eating and Physical Activity Trial (UPBEAT), a randomised controlled trial of a behavioural intervention (diet and physical activity) in obese pregnant women. As the trial, did not show a reduction in the primary outcomes; incidence of gestational diabetes and delivery of a large for gestational age neonate, the dataset was treated as a cohort.

Modifiable maternal risk factors including early pregnancy measures of maternal adiposity, were linearly associated with neonatal adiposity. An independent association with maternal birthweight and neonatal adiposity was also identified. Mode of early infant feeding (breast, formula and mixed feeding) and measures of general appetite influenced measures of infant growth and body composition at 6 months in offspring born to obese women from the UPBEAT cohort; providing evidence of a potential target for intervention within a sensitive window of opportunity.

To address mechanistic pathways underpinning the associations between *in-utero* exposures and offspring adiposity, assessment of the cord blood metabolic profile was undertaken including measurement of candidate biomarkers and metabolome. A novel relationship between cord lysophosphatidylcholine with neonatal and infant anthropometry was identified, which was associated with maternal fasting glucose in late second trimester.

Maternal fasting glucose in late second trimester was also found to partially mediate the effect of maternal parity and early pregnancy adiposity with neonatal adiposity.

The UPBEAT intervention was associated with a reduction in a measure of offspring central adiposity, which was mediated through changes in antenatal diet and gestational weight gain instigated by the intervention. This provides evidence, within a randomised control trial setting that a behavioural intervention may potentially reduce offspring adiposity.

Chapter 1 Introduction

1.1 Childhood obesity

1.1.1 Epidemiology

Globally, 41 million children under the age of 5 years are currently estimated to be overweight or obese (Ng *et al.*, 2014; Poston *et al.*, 2016) (Figure 1.1). In a report from 2015 it was suggested that in the UK, 24.4% of children aged between two and five years are overweight (defined as >85th centile weight for length z-scores) (de Onis *et al.*, 2010; van Jaarsveld and Gulliford, 2015). There is now evidence to suggest that many of these obese children were born large for gestational age (LGA) or macrosomic (birthweight >4kg) (Cunningham *et al.*, 2014). A population study, undertaken in the United States of America (USA) in children (n=21,260) aged 0-14 years, found that 36% of childhood obesity from 5 to 14 years occurred in macrosomic neonates (Cunningham *et al.*, 2014). This study also demonstrated that obesity tracked through childhood, with an incidence at 14 years of age, 4 times higher amongst overweight children at 5 years of age (Cunningham *et al.*, 2014); suggesting that obesity in adolescence is associated with birthweight and subsequently childhood obesity.

Childhood obesity is implicated with numerous adverse health consequences, summarised below.

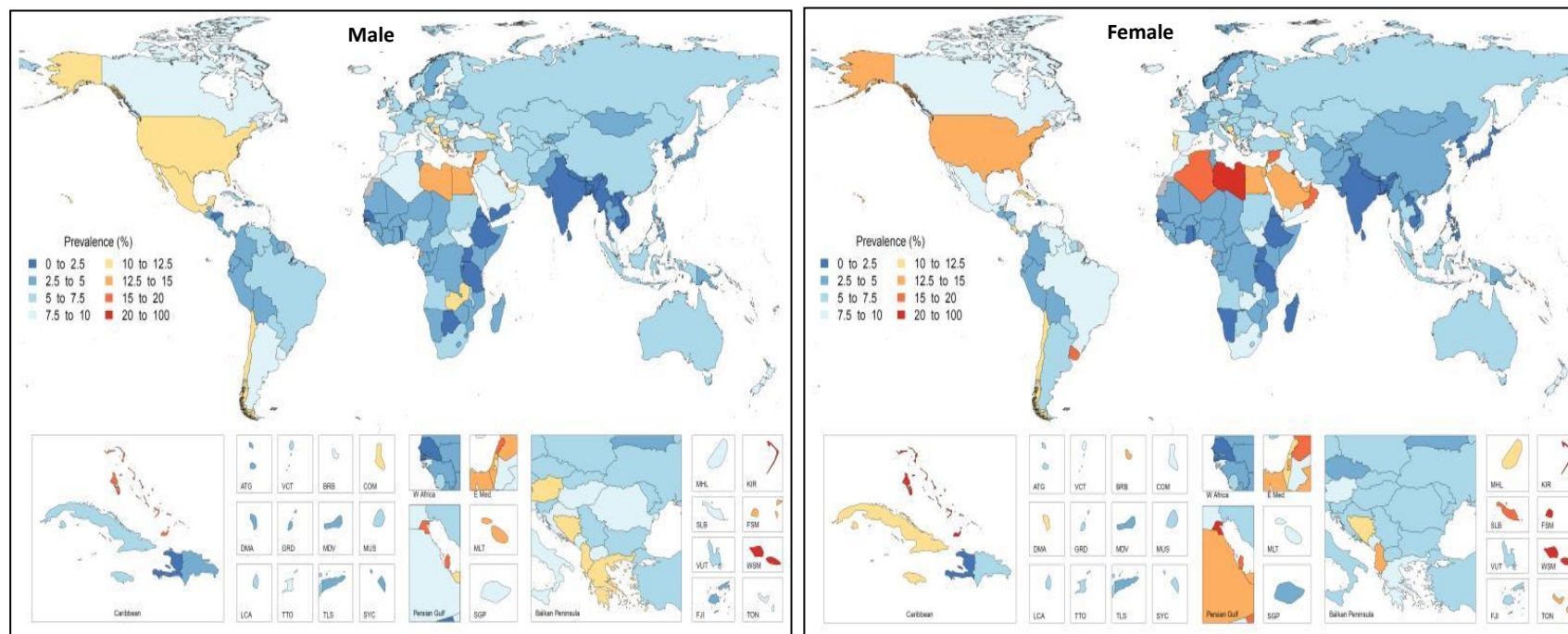


Figure 1.1: Age standardised global prevalence of obesity defined by the International Obesity Task Force Criteria for male and females aged between 2-19 years of age.

Age standardised prevalence rates were calculated using the standard population distribution based on the average country distribution by age determined by World Population Prospects 2012 Revision. Source: Ng et al. Global, Regional and National Prevalence of Overweight and Obesity in Children and Adults 1980-2013: A Systematic Analysis. *Lancet* 2014;384:766-781 (Ng et al., 2014).

1.2 Health consequences of childhood obesity

The consequences of childhood obesity can be classified into immediate adverse health (childhood consequences) and those that persist throughout the life course (adulthood consequences).

1.2.1 Childhood consequences

Childhood obesity is associated with a wide range of immediate psychological and psychosocial ill health including depression, anxiety and low self-esteem (Reeves *et al.*, 2008). Population level analysis, from Booth *et al.*, found that children who are obese at 11 years of age had significantly reduced academic attainment at 16 years of age in comparison to non-obese children (Booth *et al.*, 2014). If this relationship between childhood obesity and academic attainment were found to be causal, this may impact upon later employment and subsequent socioeconomic status (Johansson and Sundquist, 1999). Paediatric obesity is associated with concurrent prevalence of fatty liver disease, asthma and allergy (Dietz, 1998; Ebbeling *et al.*, 2002). Health care costs, are also higher amongst overweight and obese children, due to an increased frequency of utilisation of healthcare services, hospitalisations and prescribed medications in comparison to those children of normal weight and of corresponding age (Hayes *et al.*, 2016).

1.2.2 Adulthood consequences

Overweight and obese children are more likely to have risk factors increasing their susceptibility to later disease as adults. Childhood obesity increases not only susceptibility to later obesity but also insulin resistance and cardiovascular disease throughout the life course (Lawlor, 2013). Within an Israeli population study including 2,298,130 adolescents (followed up for 42,297,007 person-years) aged between 16 and 19 years, obesity during adolescence (defined as BMI $\geq 95^{\text{th}}$ percentile) was associated with a substantially increased

risk of mid-life cardiovascular mortality, in particular from coronary artery disease (17.7% associated cardiovascular mortality (95% CI 14.9% to 20.7%), following adjustment for age, birth year, sex, education, socioeconomic origin and country of origin (Twig *et al.*, 2016) (Figure 1.2). A dose-response relationship is evident between the length of time an individual is obese and subsequent risk of adult onset cardiovascular disease and all-cause mortality (Aune *et al.*, 2016).

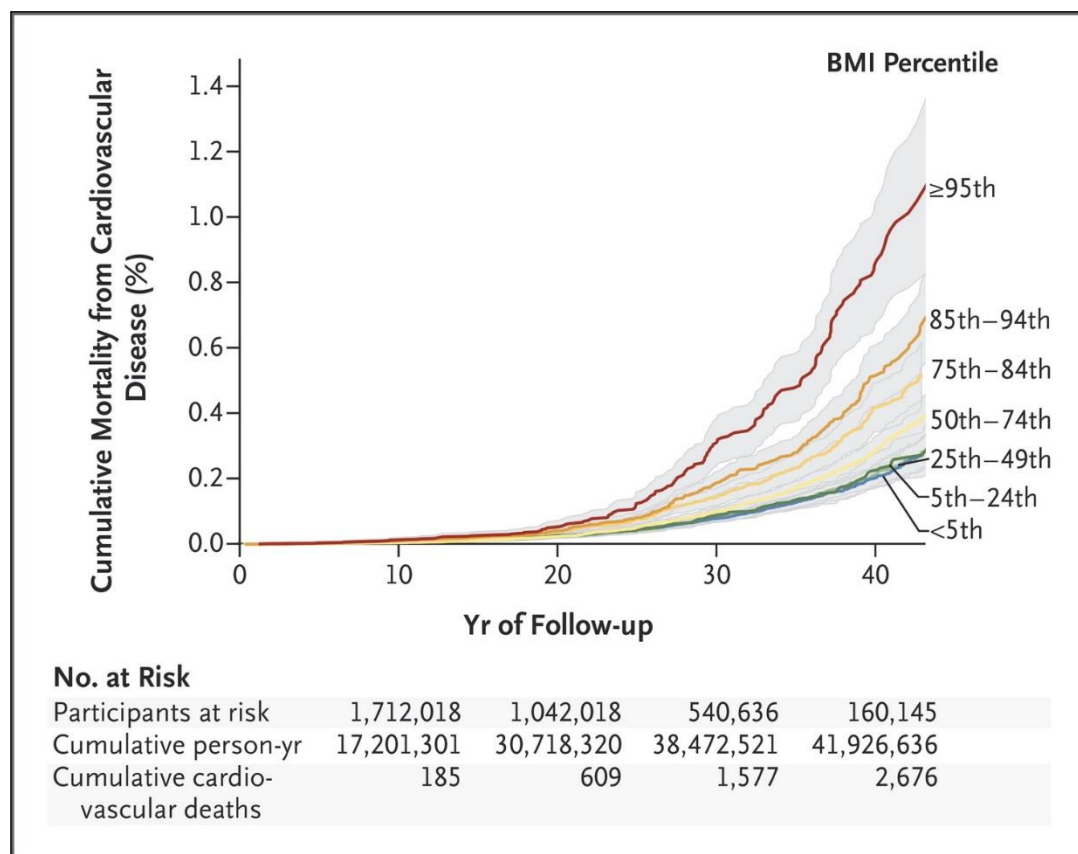


Figure 1.2: Body mass index during adolescence and subsequent risk of cardiovascular mortality.

2,298,130 participants followed-up for 44 years according to the percentile of BMI during adolescence (measured at 16-19 years). Grey shading indicates 95% confidence intervals and all models were adjusted for sex, age at examination, birth year, education, socioeconomic status, country of origin and height. Within this study, there were 2,918 deaths from cardiovascular causes including coronary artery disease, stroke and sudden death of unknown cause. Source: Twig *et al.* Body Mass Index in 2.3 Million Adolescents and Cardiovascular Death in Adulthood. *New England Journal of Medicine* 2016;374:2430-2440 (Twig *et al.*, 2016).

1.3 Causes of childhood obesity

The increasing prevalence of obesity is thought to be due to an energy imbalance secondary to energy intake exceeding energy expenditure. Obesity is a complex, multifactorial trait, composed of interlinked genetic and environmental components (Aguilera *et al.*, 2013). The global increase in obesity over the last two generations from 15% to 33.9% globally, suggests that although genetic variants may play a contributing role, an increasing emphasis should now be placed on the potential role of environmental factors.

1.3.1 Genetic factors associated with childhood obesity

Within the adult population, twin studies have demonstrated that concordance for fat mass is higher in monozygotic twins in comparison to dizygotic twins suggesting a genetic component for the development of adiposity (Silventoinen *et al.*, 2009). Genome wide association studies (GWAS) within adult populations (n=249,796), have demonstrated 32 confirmed loci, explaining 1.45% of inter-individual variation in BMI, where the Fat Mass and Obesity (FTO) gene accounted for the largest proportion of variance (0.34%) (Speliotes *et al.*, 2010; Fall and Ingelsson, 2014). Using stimulation studies, Speliotes *et al* determined that for each additional risk allele, there was an associative increase in BMI by 0.17 kg/m² (Speliotes *et al.*, 2010).

Evidence for a potential genetic contribution in the development of childhood obesity, has arisen from well conducted twin and sibling studies, which have suggested that 40-70% of the inter-variation associated with obesity may be of a genetic origin (Maes *et al.*, 1997). A landmark study, conducted by Whitaker *et al*, found that the development of mid-childhood obesity at 10-14 years of age was associated with a 4-5 fold increased risk if both parents were obese, in comparison to those with normal weight parents (Whitaker *et al.*, 1997). Furthermore, within a twin population study including 2,508 male twin sets, clustering of genetic determinants predisposing to hypertension, diabetes and obesity were found in

31.6% monozygotic pairs and 6.3% dizygotic pairs (Carmelli *et al.*, 1994), providing consistent evidence of a genetic involvement in the development of adulthood cardiometabolic disease (Aguilera *et al.*, 2013).

GWAS studies, have recently provided robust evidence for the role of variant genes in the development of childhood obesity. The Avon Longitudinal Study of Parents and Children (ALSPAC) identified associations with genetic variants within the FTO gene, the Melanocortin 4 receptor (MC4R) gene and transmembrane protein genes as assessed in cord leucocytes, with mid-childhood body mass index (BMI) at 11 years of age (Sovio *et al.*, 2011). The study further identified a relationship with a *rs9939609* variant which was associated with an accelerated adiposity rebound in early childhood but also increased BMI by 0.1 kg/m² per allele from birth to 13 years of age (Sovio *et al.*, 2011). Development of an ‘obesity allele risk-score’ with inclusion of these causative genetic variants, den Hoed *et al* demonstrated that offspring (n=7,146) with a raised obesity risk score were heavier and longer at 6 weeks of age, findings of which were replicated in the Severe Childhood Onset and Obesity project (n=1038) (den Hoed *et al.*, 2010). A meta-analysis of 2.7 million single nucleotide polymorphisms (SNPs), from 14 included studies, consisting of 8,318 normal weight (<50th percentile of BMI) and 5,530 obese children (95th percentile of BMI) confirmed previous findings of a role of FTO, transmembrane protein gene (TMEM18), Pro-opiomelanocortin (POMC), Melanocortin 4 receptor (MC4R) and others, as well as a novel role for HOXB5 (implicated in regulating gut function) and OLFM4 (involved in cell adhesion and motility) with the development of childhood obesity (Figure 1.3) (Bradfield *et al.*, 2012).

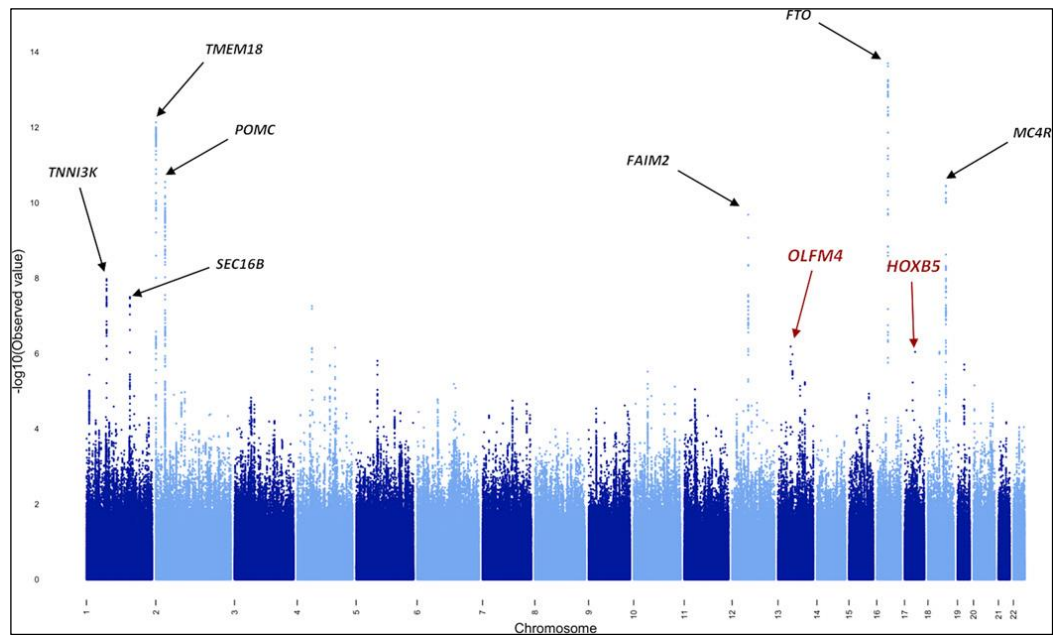


Figure 1.3: Manhattan plot of the genome wide association meta-analysis of childhood obesity.

Each locus was found to achieve genome wide significance ($p < 5 \times 10^{-8}$). Novel loci identified in this study are indicated in red text. Source: Bradfield JP *et al.* A genome-wide association meta-analysis identified new childhood obesity loci. *Nature Genetic* 2012; 44:526-531 (Bradfield *et al.*, 2012)

These findings collectively suggest that as few as 5% of cases of childhood obesity are directly caused by a defective gene due to the observed small effect estimates (Bouchard, 2009). The development of childhood obesity is thought to result from an acquired predisposition favouring obesogenic behaviours within a high-risk obesity environment (Bouchard, 2009). Studies investigating the role of genetic factors in the development of childhood obesity have provided mechanistic insight between the interaction of environmental risk factors and genetic susceptibility to obesity related traits (Frayling *et al.*, 2007). However, these relationships are potentially confounded by environmental factors shared amongst family members. Insight into the interactions between key genetic and causative environmental factors, may provide additional targets for intervention within early life for the prevention of later obesity (Dodd *et al.*, 2016).

1.3.2 *Childhood lifestyle behaviours*

1.3.2.1 Diet

Observational and randomised controlled studies (RCTs), have demonstrated that formula feeding promotes rapid early postnatal growth, associated with the development of later childhood obesity, in comparison with exclusive breastfeeding (Kramer, 1981; Ong *et al.*, 2006). However, the effect sizes are small and are discussed in detail in *Section 1.3.12:*

Postnatal feeding.

A high saturated fat diet, increased consumption of sugar-sweetened beverages and caloric intake have been consistently associated with the development of childhood obesity. As food preferences are determined in early childhood, it is suggested that poor diet may increase susceptibility to later life obesity. However, assessment of secular trends in dietary composition over the last twenty-five years using data from the Nationwide Food Consumption Survey have identified a constant calorie intake, placing an increasing emphasis nutrient composition (Evert *et al.*, 2014)

A relationship between increased consumption of sugar-sweetened beverages and increased weight gain in children has been consistently demonstrated, as recently observed in a systematic review (Malik *et al.*, 2013). In a more recent study, a post-hoc analysis of a blinded RCT replacing sugar-sweetened with sugar free beverages over an 18 month period in children 7-10 years of age, consumption of sugar-sweetened beverages was associated with a significant increase in weight particularly in those children overweight and obese at baseline (Katan *et al.*, 2016). In another recent report, intakes of sugar-sweetened beverages were found to be highest in overweight and obese children in a cross-sectional study of 1075 children aged 8-11 years, with an increase in consumption of 330ml/day being associated with a 0.51kg/m² (95% CI 0.21 to 0.81) rise in BMI (Harrington *et al.*,

2016). The prominence of ‘fast-food’ within children’s diet has quadrupled, as observed in a USA population study in 6212 children aged between 4-19 years (Bowman *et al.*, 2004). Indeed increased availability and access to processed food is thought to play a significant role in the increasing incidence of obesity (Ebbeling *et al.*, 2002), highlighting the need to modify dietary composition in children to reduce the incidence of obesity.

1.3.2.2 Physical activity

Physical inactivity defined as increased sedentary behaviour, is associated with increased risk of childhood obesity (Tremblay and Willms, 2003; LeBlanc *et al.*, 2015). Physical inactivity has been shown to be linearly associated with an increase in screen/ television viewing time in a study including 5,844 children (mean age 10.4 years) (LeBlanc *et al.*, 2015), and which has been shown to be associated with an increase in energy and total dietary fat intake (Dietz and Gortmaker, 1985; Hill and Peters, 1998). A population study in the USA (n=284, 675), found that 79% of children watched television >1hr daily. The time spent watching television was linearly associated with BMI in adolescents and children, exhibiting a dose response relationship (Greaves *et al.*, 2011). The relationship was demonstrated as causal, in an RCT assessing the provision of educational aids focused on reducing television and video game use in 192 school children (mean age 8.9 years) (Robinson, 1999). Children randomised to the intervention arm had a reduction in BMI (adjusted relative difference -0.45 kg/m² (95% CI -0.73 to -0.17), triceps skinfold thicknesses (SFT) (-1.47mm (95% CI -2.41 to -0.54)) and waist circumference (-2.30cm (95% CI -3.27 to -1.33)), suggesting a potential causal relationship between physical activity and childhood obesity (Robinson, 1999).

While there is little doubt that a high fat, western style diet combined with a reduction in physical activity are strong contributors (Robinson, 1999; Moreno and Rodríguez, 2007); early-life exposures may also play a causative and persistent role in the developmental of

childhood obesity, a concept integral to the Developmental Origins Health and Disease Hypothesis (DoHaD).

1.3.3 The DoHaD hypothesis (developmental programming)

Observational data and animal studies, have highlighted associations between alterations in the *in-utero* environment and increased susceptibility to obesity and adverse cardiometabolic profiles in adulthood. A combination of antenatal and postnatal factors have been implicated in the development of infant and childhood obesity including, in the first instance, maternal undernutrition (Barker *et al.*, 1989; Whitaker *et al.*, 1997; Hattersley and Tooke, 1999) and more recently maternal obesity, excessive gestational weight gain (GWG), gestational diabetes (GDM) and early life feeding and growth trajectories (Nelson *et al.*, 2010). This has led to numerous studies exploring these associations and potential contributory mechanisms, with the ultimate objective being the development of interventions in early in life to reduce the risk of offspring obesity.

Studies in experimental animals were amongst the first to propose that the in utero environment including maternal size and nutrition could be play a role in future development. The classic Shire horse/Shetland pony breeding experiments of McCance and Widdowson (McCance and Widdowson, 1974), for example demonstrated the role of intrauterine restraint on future growth trajectory. Work by Dubos et al in 1966 identified a consistent trend between the earlier maturation of children attributable to environmental influences in early life. His animal models focused primarily on early postnatal life as a critical window for environmental exposures to influence development. Within a genetically identical mice model, Dubos et al demonstrated that growth trajectories in early life are determined at birth (Dubos et al, 1966). These experimental animal studies showed proof of principle of the developmental programming hypothesis but predated the description of the hypothesis based on the evidence derived from human population studies

by Barker et al (Barker, 2002; Almond and Currie, 2011) The Barker hypothesis originated from studies within historical population cohorts assessing the influence of *in-utero* exposure to maternal undernutrition. Low birthweight was found to be associated with inadequate nutrition *in-utero* 'programming' the fetuses' susceptibility to future cardiovascular and metabolic disease (Barker, 2002). Whilst a reduction in birthweight is evident at birth, it is apparent that more subtle effects within the *in-utero* environment may have latent effects on the offspring phenotype. A series of observational studies derived from the Dutch "Hunger Winter" determined the underlying effects of *in-utero* programming including the observation that famine exposure *in-utero* was associated with a two-fold increase in the incidence of obesity at 18 years of age in offspring without a change in birthweight (Ravelli *et al.*, 1976; Murray *et al.*, 2015). Intrauterine insults from normal development during the critical phases of growth and development have been associated with permanent functional and structural changes in certain organs including liver and kidney and cellular function within adipocytes, myocytes, nephrons, neurons and pancreatic beta cells (Vickers *et al.*, 2000; Bagby, 2007).

Barker and Hales subsequently formulated the 'Thrifty Phenotype' hypothesis, to account for the observed correlations between birthweight and development of type 2 diabetes (Hales and Barker, 2001). It was hypothesised that fetuses experiencing poor maternal nutrition *in-utero* are prone to permanent modifications in glucose metabolism, consequently associated with an increased risk of cardiometabolic disease. It was proposed that fetuses exposed to poor maternal nutrition undergo plasticity adaptive responses which conserve energy to protect fetal survival (Desai *et al.*, 2015). However, when offspring are faced with an affluent postnatal environment, this adaptive response may increase the velocity of early-life growth, which will lead to loss of cellular function in already compromised tissues including the pancreas and heighten insulin resistance (Figure 1.4) (Desai *et al.*, 2015).

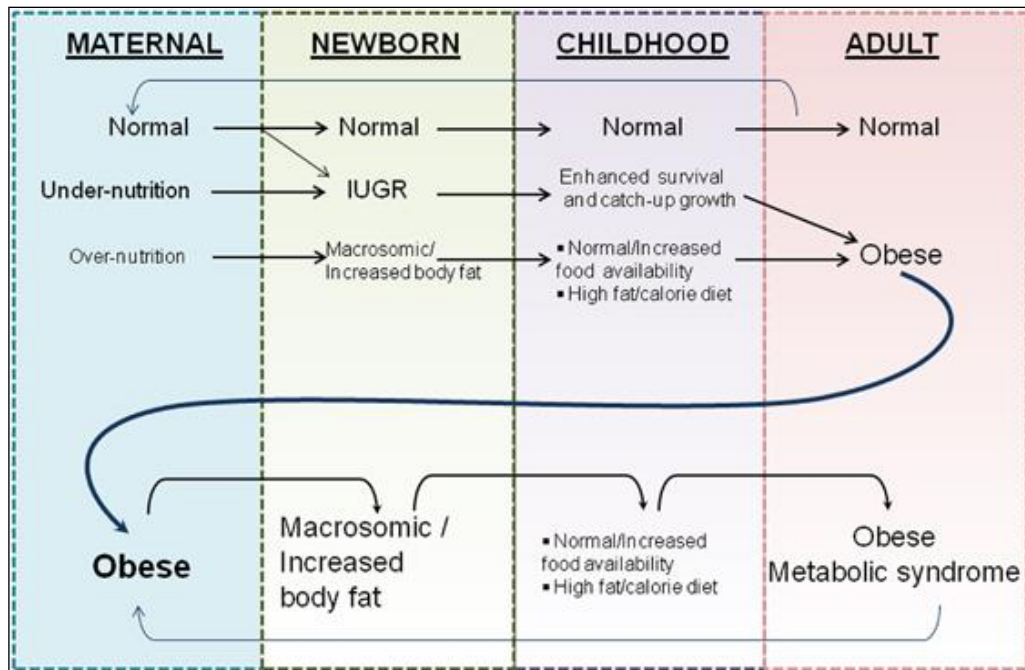


Figure 1.4: Proposed role of *in-utero* programming contributing to the increased prevalence of obesity and metabolic syndrome.

Source: Desai M *et al.* Epigenomics, Gestational Programming and Risk of Metabolic Syndrome. *International Journal of Obesity* 2015;39:633-41 (Desai *et al.*, 2015).

Robust evidence from observational and birth cohort studies have identified that the trajectory for later obesity may be established by the age of 3-5 years (Stuart and Panico, 2016). The inference being that intervening prior to pre-school may provide one approach to curb the increasing incidence of childhood obesity. Observational and experimental animal studies suggest that the offspring's response to obesogenic environmental exposures within the postnatal period, specifically feeding practices, may be determined by intrauterine exposures and early postnatal development (Bateson *et al.*, 2004; Parlee and MacDougald, 2014). The upward crossing of growth centiles in early life, specifically rapid weight gain, resulting in increased fat deposition within the first 6 months (Rogers, 2003),

has been shown to be associated with increased risk of later obesity and an adverse cardiometabolic risk profile in adolescence and early adulthood (Ong and Loos, 2006; Fall *et al.*, 2008; Taveras *et al.*, 2009; Perng *et al.*, 2016; Voerman *et al.*, 2017). Others have shown that accelerated weight gain and growth in the first 6 months of life is associated with a 3.4 increased risk of overweight at 7 years of age (Stuart and Panico, 2016). A rapid increase in weight for length z-scores has also been associated with raised hypertension and adiposity in adolescents (Taveras *et al.*, 2009; Perng *et al.*, 2016). Furthermore, 4 discrete BMI trajectories have been identified from birth to 3.5 years of age, characterised as low, intermediate or high accelerating growth traits, evident at 6 months of age, which demonstrated a linear graded effect on the risk of overweight and obesity at 9 years of age (Giles *et al.*, 2015).

1.3.4 *In-utero determinants of offspring adiposity*

In light of the increasing obesity epidemic, the fundamentals of the Developmental Programming hypothesis have been adapted to include exposure to maternal over-nutrition *in-utero* and subsequent offspring health. The concept that maternal “overnutrition” could lead to excess fetal growth and adiposity was derived from the knowledge that maternal hyperglycaemia stimulates fetal hyperinsulinemia, a major driver of fetal adiposity, as described by the Pedersen hypothesis (Macfarlane and Tsakalakos, 1988; Catalano *et al.*, 1995; Barker, 2002; Catalano *et al.*, 2009; Catalano and Hauguel-De Mouzon, 2011). The maternal factors associated with dysglycaemia include dietary behaviours, pre-pregnancy obesity, GWG and GDM, all of which independently, or through interactive pathways, contribute to offspring adiposity as illustrated in Figure 1.5.

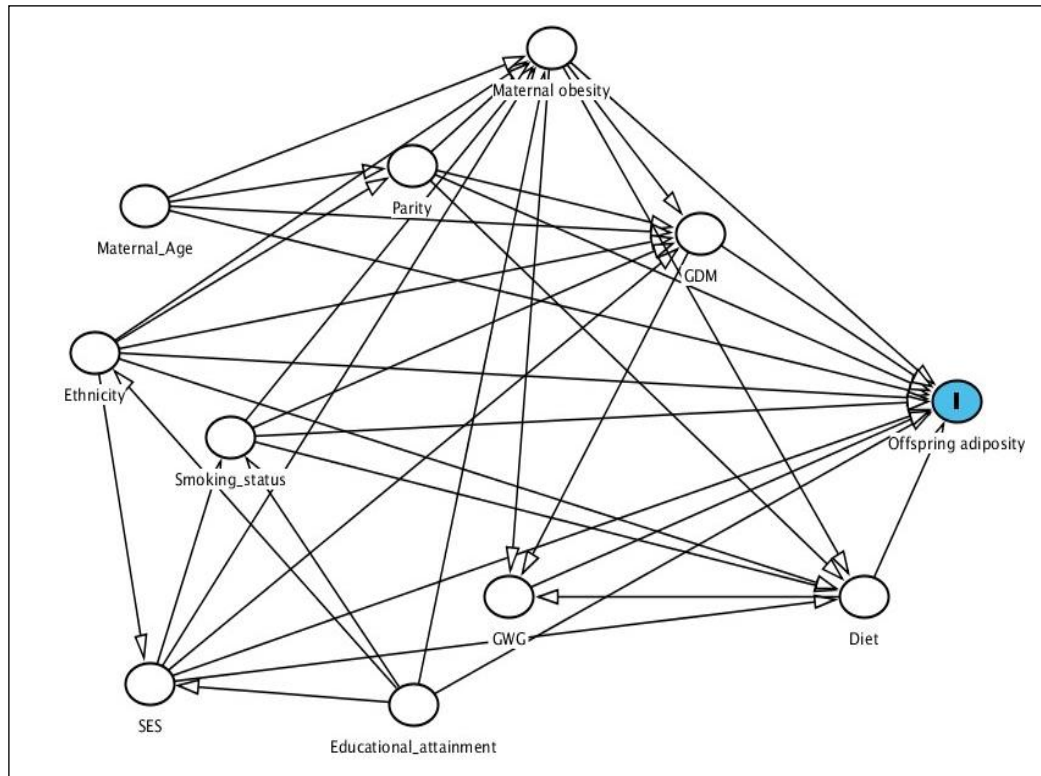


Figure 1.5: Associations between maternal dietary behaviours, pre-pregnancy obesity, gestational weight gain, gestational diabetes and other potential confounding factors implicated in the development of offspring adiposity.

Abbreviations GDM- Gestational diabetes, GWG-Gestational weight gain, SES- Socioeconomic status (deprivation).

1.3.5 Normal, healthy pregnancy

An understanding of normal metabolic and physiological changes in relation to fetal growth is essential to understand the mechanistic pathways between the *in-utero* maternal environment, fetal growth and subsequent infant obesity, as described below.

1.3.5.1 Normal fetal growth

There are three phases to normal fetal growth during human pregnancy. Figure 1.6 summarises normal fetal estimated weight, length and head circumference development throughout gestation. During early gestation, implantation, placentation and embryogenesis occurs within the first 2-3 weeks of pregnancy (Zhang *et al.*, 2010). After an early

histotrophic phase of embryonic growth, placental nutrient transfer is established in the early 2nd trimester of pregnancy (Bauer *et al.*, 1998). During the second trimester, growth, development and function of critical organs including the pancreas, brain and kidneys is established. By 20 weeks, the fetus has reached 20% of the potential weight and 60% of its total length at term (Zhang *et al.*, 2010). During the early 3rd trimester, there is a phase of accelerated fetal growth and adiposity (Dunger *et al.*, 2006).

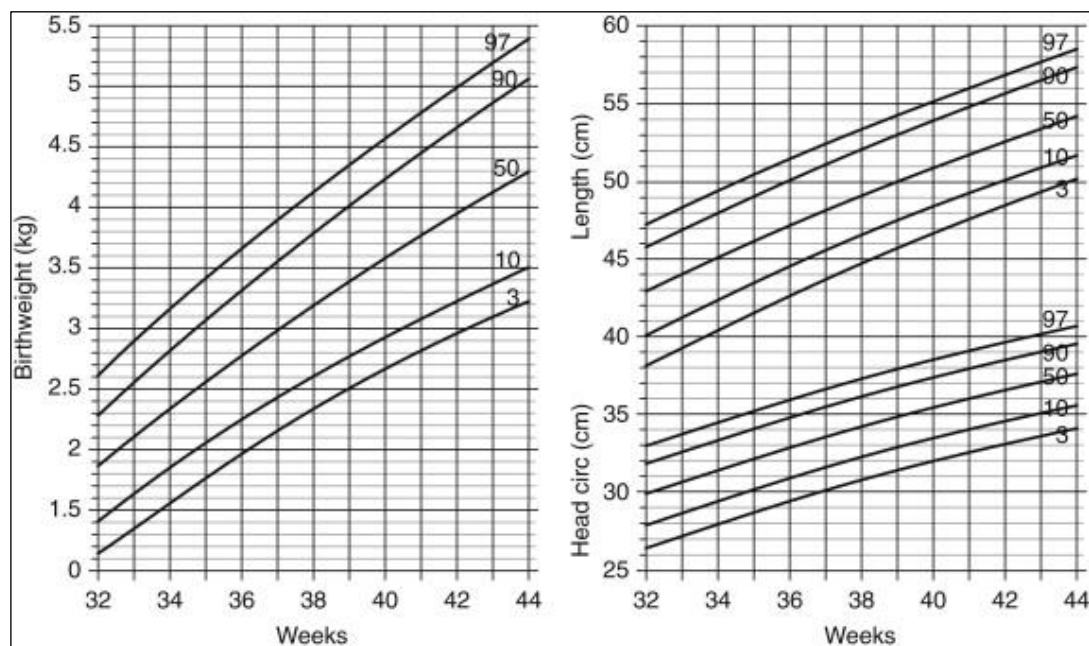


Figure 1.6: Normal development of fetal weight, length and head circumference trajectories as a function of gestational age in normal pregnancies.

Source: Smith JB. Chapter 25- Initial Evaluation: History and Physical Examination of the Newborn. Avery's Diseases of the Newborn (9th edition). 2012;1:277-299 (Smith, 2012).

1.3.5.2 Physiological metabolism of normal pregnancy

To establish normal growth and development of the fetus, complex interactions and alterations in maternal fat deposition, calorie intake and basal metabolic rate occur, as summarised in Figure 1.7. Early pregnancy is categorised as an anabolic phase, thereby increasing maternal fat mass through enhanced glycogenesis, lipogenesis and protein storage (Lain and Catalano, 2007). The third trimester is associated with a predominantly catabolic phase, coinciding with increased fetal growth. During this phase, maternal

circulating glucose, free fatty acids, triglycerides and cholesterol (including lipoprotein components) increase to support the demands of the growing fetus (Godfrey *et al.*, 1996a; Lain and Catalano, 2007). In addition to these maternal adaptations, maternal insulin secretion progressively increases throughout pregnancy with a subsequent decrease in insulin sensitivity (Rosso, 1988). These changes are associated with a continual rise of maternal fasting glucose throughout gestation, but in the context of a normal pregnancy, the thresholds for diagnosis of GDM are not exceeded (Bleicher *et al.*, 1964; Lain and Catalano, 2007).

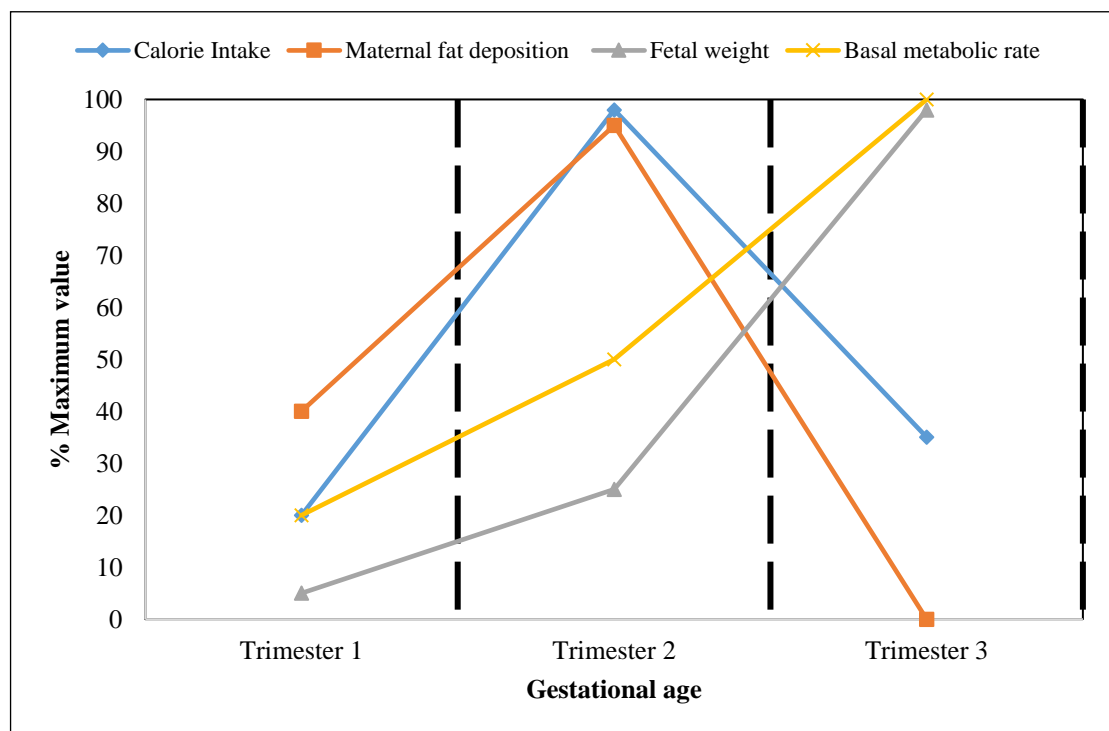


Figure 1.7: Maternal metabolic changes and fetal weight during normal pregnancy.

Source: Adapted from Rosso, P. Regulation of Food Intake during Pregnancy and Lactation. Annals Academic Science, 1988; 499: 191 (Rosso, 1988).

1.3.6 The global obesity epidemic in the context of maternal obesity

1.3.6.1 Global prevalence of maternal obesity

Obesity is defined as a BMI $\geq 30 \text{ kg/m}^2$. The increase in obesity is a major health concern, having reached epidemic proportions with the prevalence having doubled globally in the

last twenty years. The World Health Organisation (WHO) has estimated that 300 million of the 1.5 billion adults in the world are currently defined as overweight or obese (WHO, 2012). Furthermore, the Non Communicable Disease Risk Factor Collaboration reported a 14.9% incidence of obesity in women, estimated from an international population of 19.2 million adults (NCD-RisC, 2016).

Obesity rates are greatest within high income countries, with a 25% incidence of maternal obesity in the UK (Poston *et al.*, 2016) (Figure 1.8). Demographic forecasts demonstrate that the prevalence of female obesity will exceed 21% globally by 2025, based on the post-2000 trends (NCD-RisC, 2016; Poston *et al.*, 2016).

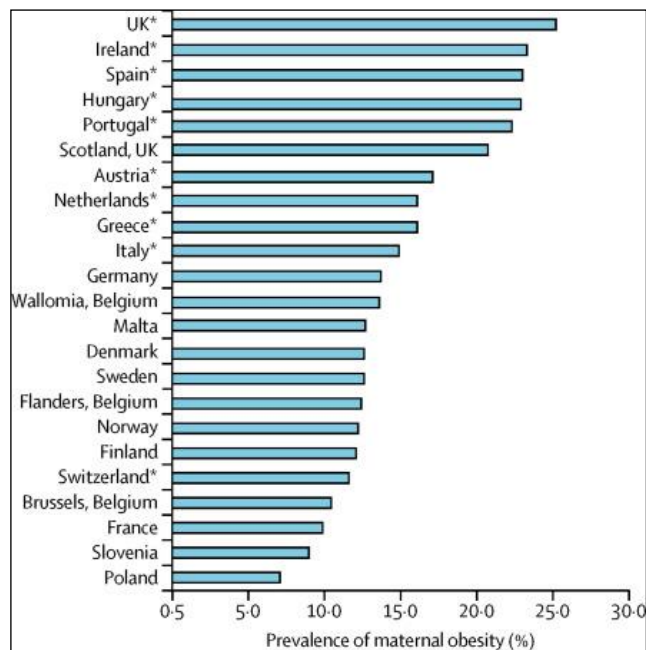


Figure 1.8: Distribution of maternal obesity from the EURO-Peristat database.

*From WHO database (2009); includes all women aged 20 years or above as proxy for maternal obesity.
Source: Poston *et al.* Preconceptional and Maternal Obesity: Epidemiology and Health Consequences. *Lancet Diabetes and Endocrinology*, 2016;4:1025-1036 (Devlieger *et al.*, 2016; Poston *et al.*, 2016).

A linear relationship exists between increasing pre-pregnancy BMI and the risk of many antenatal complications (O'Brien *et al.*, 2003; Marshall *et al.*, 2014). Maternal obesity is an independent risk factor for adverse outcomes in pregnancy; including pre-eclampsia, GDM and delivery of an LGA infant (Chu *et al.*, 2007; Yu *et al.*, 2013) all of which have been associated with adverse long-term health consequences for the offspring. Obese women are

more likely to experience complications during labour, including higher rates of induction of labour and caesarean sections (Heslehurst *et al.*, 2010). A high maternal BMI is also an important risk factor for intrauterine growth restriction (IUGR) (Ramachenderan *et al.*, 2008), associated with the development of later cardiovascular and metabolic disease in the offspring (Barker *et al.*, 1993; Barker, 1997; Demicheva and Crispi, 2014).

1.3.6.2 Maternal obesity and offspring obesity and body composition

Observational studies have demonstrated that a higher pre-pregnancy BMI is associated with raised BMI in the adult offspring, independent of socioeconomic and dietary confounding (Yu *et al.*, 2013). For example, within a large multi-ethnic observational cohort, Lemas *et al.*, reported that maternal BMI prior to pregnancy was associated with increased umbilical cord blood leptin, glucose and reduced high density lipoprotein cholesterol (HDL-c) at delivery with neonatal adiposity (n=753), a metabolic profile favourable with the development of obesity in adulthood (Lemas *et al.*, 2015).

A report from a sub-group from the Generation R population cohort from the Netherlands assessed the influence of maternal obesity on childhood outcomes at 4 years of age. Maternal obesity was associated with an increased odds of developing childhood obesity (adjusted odds ratio (AOR) 5.02; 95% CI 2.97 to 8.45) (defined as $\geq 85^{\text{th}}$ centile, International Task Force reference population) independent of socioeconomic deprivation and poor lifestyle behaviours (Gaillard *et al.*, 2013). The ALSPAC cohort assessed 8234 women using self-reported pre-pregnancy weight and offspring were followed up to 7 years where obesity was defined as BMI greater than the 95th percentile (equivalent to a standard deviation of ≥ 1.96) adjusted for age and gender. Pre-pregnancy obesity was independently associated (adjusted for maternal education, offspring energy intake at 3 years and offspring sex), with a significant increased risk of childhood obesity at 7 years (AOR 4.25; 95% CI 2.86 to 6.32) (Reilly *et al.*, 2005). These finding were replicated in the population

cohort study from the USA (Nurses' Health Study II and the Nurses' Mother cohort) where maternal obesity (n=26,506) was associated with a 6.1 fold increased risk of obesity in the offspring (Stuebe *et al.*, 2009).

However, childhood BMI has been shown to poor predictive measure of later clinical outcomes. Distinguishing the deposition of adiposity in relation to *in-utero* exposures may provide insight into the development of later adverse clinical outcomes. In a prospective cohort study of a subgroup of 216 women from the Southampton Women's Survey (UK), data on maternal weight and skinfold measurements from pre-pregnancy to 34 weeks' gestation were collected. Offspring adiposity was assessed in 9 year old offspring using the fat mass index (FMI) measured by dual energy X-Ray absorptiometry (DEXA). For each standard deviation increase in maternal pre-pregnancy BMI there was an increase in offspring FMI by 0.26 (95% CI 0.04 to 0.48) in males and 0.42 (95% CI 0.29 to 0.56) in females (Gale *et al.*, 2007). These findings were further replicated in a large meta-analysis (including n=20 randomised controlled and cohort studies) which assessed the effect of pre-pregnancy maternal BMI on offspring fat-free mass, fat mass and % body fat. Maternal overweight and obesity was demonstrated to be positively associated with increased offspring adiposity (fat mass 0.38 kg, 95% CI 0.26 to 0.50; fat-free mass 0.18kg, 95% CI -0.07 to 0.42), in comparison to offspring born to normal weight women (Figure 1.9) (Castillo-Laura *et al.*, 2015).

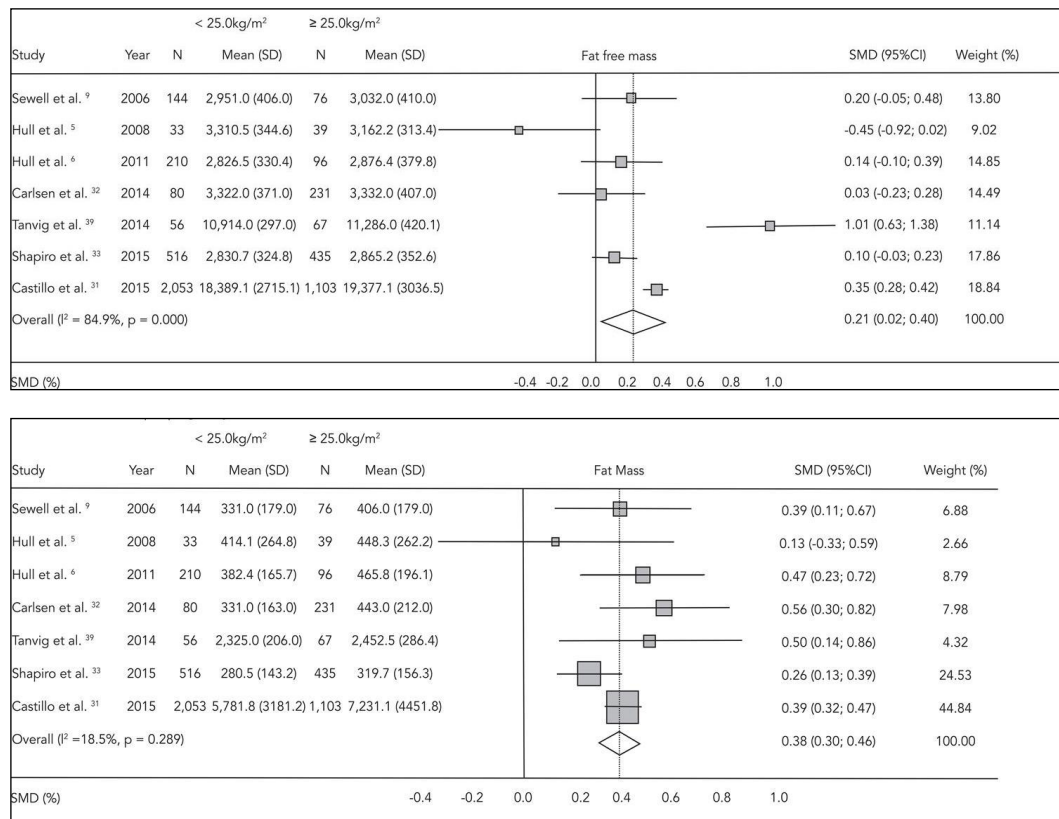


Figure 1.9: Standardized mean differences in offspring body composition (fat free mass and fat mass) stratified by maternal pre-pregnancy BMI category.

Weights are from random effects analysis. 95%CI: 95% confidence interval; SMD: standard mean difference (%). Source: Castillo-Laura H et al. Maternal Obesity and Offspring Body Composition by Indirect Methods: A Systematic Review and Meta-Analysis. *Cadernos de Saude Publica* 2015;35:20173-92 (Castillo-Laura et al., 2015).

A study from the Generation R cohort, assessing the individual and combined exposure of maternal and paternal (n=4871) associations of childhood BMI has enabled clarification of a potential role of the maternal *in-utero* environment. Six year old offspring born to obese mothers had an increased risk of both being overweight in childhood (OR 3.84; 95% CI 3.01 to 4.90) and of an increased cardiometabolic risk score including android fat mass percentage ≥75th percentile; systolic or diastolic blood pressure ≥75th percentile; high-density lipoprotein cholesterol ≤25th percentile or triglycerides ≥75th percentile; and insulin ≥75th percentile (OR 3.00; 95% CI 2.09 to 4.34) in comparison to those of normal weight women (Gaillard *et al.*, 2014b). Furthermore, this association was stronger with

maternal pre-pregnancy BMI than paternal BMI, providing further support for the *in-utero* origins of adverse health in later life.

Whilst observational studies have demonstrated the association of pre-pregnancy BMI and childhood obesity, RCTs and the use of Mendelian randomisation (MR) methodology are required to determine causality of these associations. MR methodology has recently been adopted to investigate the causal effect of *in-utero* exposure to increased maternal BMI and with offspring BMI in children aged between 3-17 years in the ALSPAC and Generation R cohort. This study reported limited evidence in support of a causal linear relationship between maternal BMI and childhood obesity (Berry *et al.*, 2013). However, this methodology assumes that the genetic variants remain constant throughout the period studied and does not consider the interaction between genetic variants, early life environmental factors and the later development of obesity.

1.3.7 Gestational weight gain

Although elevated pre-pregnancy BMI has been associated with increased risk of adverse long-term offspring health, relationships between excess GWG and offspring body composition and obesity risk are less readily interpretable, partly because of differences in methods of measurements and because of the variable components of weight which can contribute to GWG (Gillman, 2012). It could be argued therefore, that the major focus on GWG which has predominated in studies assessing overweight and obese women could be misplaced due to these uncertainties (Gillman, 2012).

The fetus, placenta, adipose tissue, amniotic fluid, mammary glands and uterus all contribute to GWG. However, the fetus, placenta and amniotic fluid account for only 35% of total GWG. Maternal fluid volume expansion also makes a major contribution. Fat

accrual is another, but very variable component of GWG. Only 5% of maternal weight gain at 40 weeks' gestation constitutes as fat mass (Rasmussen and Yatkine, 2009).

The Institute of Medicine (IOM) recently published new recommendations for GWG (Rasmussen and Yatkine, 2009). The recommendations differ depending on the pre-pregnancy BMI (Table 1.1).

Pre-pregnancy BMI	BMI (kg/m²) (WHO)	Total Weight Gain Range (kg)	Rates of Weight Gain 2nd and 3rd Trimester (mean range in kg/week)
Underweight	< 18.5	12.5 – 18.0	0.51 (0.44 – 0.58)
Normal	18.5 – 24.9	11.5 – 16.0	0.42 (0.35 – 0.50)
Overweight	25.0 – 29.9	7.0 – 11.5	0.28 (0.23 – 0.33)
Obese	≥30.0	5.0- 9.0	0.22 (0.17 – 0.27)

Table 1.1: Institute of Medicine gestational weight gain recommendations by body mass index categories.

Adapted from the Institute of Medicine Guidelines on Gestational Weight Gain (Rasmussen and Yatkine, 2009).

The wide range of weight gain for each BMI category recommended reflects the imprecision of the given estimates. The current IOM guidelines were derived from limited data on six main outcomes; small for gestational age (SGA), LGA, preterm birth, caesarean birth, maternal postpartum weight retention and early childhood obesity but not further stratified by the different classes of obesity (Kapadia *et al.*, 2015). The IOM categories combine pre-pregnancy BMI and GWG. Therefore, it is difficult to distinguish the associations with offspring adverse health within the IOM categories and whether outcomes are contributed to pre-pregnancy BMI or GWG per se. There is also recognition by the IOM of the lack of evidence among socioeconomic and ethnic minority groups, potentially limiting translation to other populations (Rasmussen and Yatkine, 2009).

Excessive and below recommended GWG have been associated with adverse long term outcomes in the offspring (Villamor and Cnattingius, 2006; Kapadia *et al.*, 2015). Studies

assessing relationships between GWG and offspring body composition outcomes indicate that excessive GWG is associated with an increased risk of obesity as well as adverse cardiometabolic health (Oken *et al.*, 2007; Mamun *et al.*, 2010; Fraser *et al.*, 2011).

A recent systematic review and meta-analysis (n=12 cohort studies included) from the same group demonstrated that the offspring of women who gained above the prescribed IOM weight gain criteria, had a 40% increased risk of obesity throughout the life course (Mamun *et al.*, 2014). However, within this meta-analysis, none of the studies included, assessed associations by pre-pregnancy BMI but rather grouped the three BMI categories (normal, overweight and obese) together. Within other meta-analyses, GWG, defined above the IOM criteria (Rasmussen and Yatkine, 2009), has been associated with an increase in pre-school overweight and obesity (defined as overweight $\geq 85^{\text{th}}$ percentile and obesity BMI $\geq 95^{\text{th}}$ percentile) and with the magnitude of effect increasing from 1.5 fold to 4.4 fold, as recently shown by two meta-analyses in offspring after adjustment for offspring sex (Nehring *et al.*, 2013; Mamun *et al.*, 2014).

An important element often under-recognised is the timing of weight gain. Observational studies have suggested that the first 24 weeks of pregnancy is a crucial time period during which excessive GWG is a key risk factor for the development of GDM which has also been independently implicated in offspring obesity (Gibson *et al.*, 2012). Early weight gain has been shown to be a strong predictor of excessive GWG (Carreno *et al.*, 2012; Overcash *et al.*, 2015), rendering it a potential target for future interventions. This recommendation should be considered in light of the different components of GWG within the different trimesters, and is unlikely to reflect the relationship between measures of maternal adiposity and infant body composition. This is exemplified by one study which reported that rate of weight gain in mid-pregnancy, more likely to reflect maternal fat mass, was correlated with infant fat mass, whereas the rate of weight gain during late pregnancy,

likely to be predominately fetal was strongly associated with infant fat-free mass (Karachaliou *et al.*, 2015).

1.3.8 Gestational diabetes

Maternal obesity is an independent predictor for the development of GDM. Depending on the diagnostic criteria employed GDM affects as many as 30% of obese women in pregnancy (Poston *et al.*, 2015; White *et al.*, 2016). GDM is defined as a ‘degree of glucose intolerance, first recognised during pregnancy’ (ADA, 2010). The diagnosis is made regardless of whether glucose intolerance predated or persisted following pregnancy (ADA, 2010). At present in the United Kingdom (UK), screening tests are performed in women with significant predictors for developing GDM, including macrosomia, ethnicity, previous history of GDM and BMI (NICE, 2015). GDM for the purpose of this thesis, was defined by the International Association of the Diabetes in Pregnancy Study Group (IADPSG) criteria. This comprised of fasting blood glucose $>5.1\text{mmol/l}$, or a 1 hour glucose $>10.0\text{mmol/l}$ or a 2-hour glucose $>8.5\text{mmol/l}$ after a 75g glucose tolerance test (Metzger *et al.*, 2009).

The aetiology of GDM is heterogeneous and multifaceted, thought to result from the interaction between environmental, genetic and sociodemographic characteristics.

Although increased insulin resistance is ubiquitous in pregnancy, additional factors must drive susceptible mothers to develop GDM. The most obvious contributor is pre-existing insulin resistance associated with obesity. Obesity in pregnancy is associated with an increase in adipokines and circulating inflammatory mediators which are in turn implicated in insulin resistance (Ramsay *et al.*, 2002; Tinius *et al.*, 2015). Dyslipidaemia, found to be more prevalent amongst obese women has also been associated with reduced insulin sensitivity (Catalano 2010).

Maternal dysglycaemia and GDM are both associated with increased neonatal adiposity (Sebire *et al.*, 2001; Metzger *et al.*, 2009), having recently been demonstrated as causative through MR methodology (Tyrrell *et al.*, 2016). Using continuous glucose monitoring, Harmon *et al.* identified higher daytime and nocturnal profiles in obese women in comparison to lean women, which in turn were associated with raised neonatal body fat but not with birthweight (Harmon *et al.*, 2011). Derangements in amino acids, fatty acids, carbohydrates and lipid species as well as elevated post-prandial glucose have been implicated as playing a mechanistic role with increased neonatal birthweight and adiposity in association with increased maternal insulin resistance in late second trimester (Scholtens *et al.*, 2016) .

Other reports have also documented that offspring exposed to GDM *in-utero* have an increased risk of developing obesity in later childhood (Gillman *et al.*, 2003) and raised BMI in adulthood (Lawlor *et al.*, 2011a), in comparison to those un-exposed. Exposure to maternal GDM *in-utero* has shown to increase the odds of childhood obesity from 1.6 to 2.3 following adjustment for maternal pre-pregnancy BMI, suggesting an independent effect of GDM (Lawlor *et al.*, 2011a; Lawlor *et al.*, 2011b). The association between GDM and childhood adiposity has been reported as inconclusive in two meta-analyses (Kim *et al.*, 2011; Philipps *et al.*, 2011; Fraser and Lawlor, 2014), but has been complicated by the lack of universal screening for GDM in the included studies.

The treatment of GDM encompasses treatment with diet, metformin and/or insulin. The modality of treatment used may be indicative of the severity of the disease and has been shown to influence body composition in offspring exposed to GDM *in-utero* (Rowan *et al.*, 2011). Within obese women, this increased risk of offspring adiposity is thought to be largely mediated by adverse *in-utero* exposure to maternal glycaemic profiles (Harmon *et al.*, 2011). As adiposity, has shown to track from birth to early childhood (Mook-Kanamori

et al., 2011; Gishti *et al.*, 2014), treatment for GDM may provide a role in preventing childhood obesity.

As mentioned above, a recent, and important study has found maternal fasting glucose to be causally related to neonatal body composition, through utilisation of MR methodology (Tyrrell *et al.*, 2016). However, the associations between maternal GDM and later childhood adiposity cannot be considered causal as this associative relationship has been derived predominantly from observational studies. There is some evidence from a follow-up of RCTs in pregnancy that treatment of GDM may improve glycaemic profiles in children by improving maternal glycaemic profiles. A study of 9 year old children (n=905), whose mothers were randomised to treatment for mild GDM (including dietary advice and insulin) or to standard antenatal care at 20-28 weeks' gestation (Landon *et al.*, 2009), found no differences in offspring body composition at 5-10 years of age, but demonstrated significant improvements in fasting glucose in female offspring (Landon *et al.*, 2015).

It is important to recognise that excess early fetal growth; an independent risk for neonatal adiposity, occurs prior to the formal diagnosis of GDM at 24-28 weeks' gestation as shown recently in a prospective cohort of 4,000 nulliparous women (Sovio *et al.*, 2016), an association which was strengthened by maternal pre-pregnancy obesity. Therefore, early identification of maternal risk factors for GDM may provide a preferable approach for early preventive treatment for childhood obesity (White *et al.*, 2016).

1.3.9 Maternal demography

1.3.9.1 Maternal age and parity

Increasing maternal age is associated with adverse neonatal outcomes, which may be mediated through poor oocyte quality and increasing maternal parity; both of which have been independently associated with greater neonatal adiposity (Bottini *et al.*, 2001;

Myrskylä and Fenelon, 2012; Gaillard *et al.*, 2014a). A large population study, incorporating data from five cohorts (n=22,188), demonstrated that older maternal age (≥ 35 years) was independently associated with increased maternal fasting glucose (0.06 mmol/L, 95% CI -0.01 to 0.12), in comparison to mothers aged between 20-35 years (Fall *et al.*, 2015). However, neonatal adiposity was not determined in this population study of women living in low income countries.

Increasing maternal parity has also been associated with increasing birth weight (Catalano and Ehrenberg, 2006) and neonatal adiposity within a prospective population cohort study in the Netherlands (Generation R) (Gaillard *et al.*, 2014a) and within a rural Indian population (Joshi *et al.*, 2005); both are likely to reflect increasing maternal BMI resulting from cumulative weight retention following pregnancy (Villamor and Cnattingius, 2006), which may have an association with later childhood obesity.

1.3.9.2 Smoking status

Maternal smoking is associated with increased offspring adiposity, mediated primarily through low birthweight. Infants born to women who smoked in early pregnancy, were shown to have greater 'catch-up growth' in comparison to neonates of non-smokers (n=6,483, mean age 5-7 years of age); increasing the risk of later obesity and metabolic syndrome (Von Kries *et al.*, 2002). A meta-analysis including 44 cohort studies (n=94, 997) found that offspring born to women who smoked during pregnancy had an increased risk of obesity at 9 years (pooled AOR 1.64, 95% CI 1.42-1.90), independent of maternal socio-economic status, pre-pregnancy obesity, offspring birthweight and mode of early life feeding (breastfed vs. non-breastfed) (Ino, 2010). Causative mechanisms implicated include *in-utero* exposure to nicotine, resulting in abnormal adipocyte proliferation, and persistent changes in central autonomic pathways resulting in permanent effects on hypothalamic regulation of food intake and energy expenditure (Al Mamun *et al.*, 2006).

Recently, exposure to maternal smoking has shown to be associated with increased methylation of the MYOIG (involved in the regulation of cell elasticity) and FRMD4A gene (associated with nicotine dependence) in cord blood leucocytes and in whole blood of children at age 5.5 years (n=366) (Rzehak *et al.*, 2016), suggesting a potential epigenetic transmission of an adverse *in-utero* environment in offspring.

1.3.9.3 Socioeconomic status and educational attainment

Maternal educational attainment is linearly associated with socioeconomic status and socioeconomic deprivation increases the risk of maternal obesity and subsequent childhood obesity (Heslehurst *et al.*, 2010). Again fetal growth restriction may be implicated as infants born to women from socioeconomically deprived backgrounds, have been shown to be of reduced length at 2 months of age (difference -0.87 cm; 95% CI $-1.16, -0.58$), in comparison to those with no socioeconomic deprivation (Silva *et al.*, 2012).

1.3.9.4 Ethnicity

Maternal ethnicity, specifically an Afro-Caribbean background is associated with increased neonatal morbidity and mortality (CMACE, 2011). Maternal obesity, GDM and excessive GWG; are all independent risk factors for neonatal adiposity and are increasingly prevalent within Afro-Caribbean and Asian populations (Rosenberg *et al.*, 2005; Snowden *et al.*, 2016). The 3rd US National Health and Nutrition Examination Survey (n=2488) found that black ethnicity was associated with significantly lower birthweight in comparison to white and Hispanic ethnic groups ($p < 0.001$), in turn associated with greater fat mass in mid-childhood (Okosun *et al.*, 2000). The causative mechanisms implicated include socioeconomic factors associated with poor dietary behaviours, increased total GWG and a greater degree of dysglycaemia during pregnancy (Rodrigues *et al.*, 1999; Widen *et al.*, 2015).

1.3.10 Maternal diet

Maternal pre-pregnancy obesity is an indicator of a poor quality diet, including consumption of a high fat or ‘Western’ diet (Flynn *et al.*, 2016). There is strong evidence to suggest that maternal diet composition and quality is strongly correlated not only to maternal obesity but also to GWG and GDM (Poston, 2012). Therefore, there has been equal focus on maternal diet as on these variables in relation to childhood adiposity.

The Generation R study (n=2695), observed that adherence to a ‘healthier’ dietary pattern in pregnancy was associated with lower offspring BMI, FMI and a reduced risk of being overweight at 6 years of age, in children born to women of heterogeneous BMI (van den Broek *et al.*, 2015). However, when the results were adjusted for sociodemographic and lifestyle covariates, none of these relationships remained significant; potentially due to an interaction between maternal diet and socioeconomic status as previously reported (Falconer *et al.*, 2014). Using a data driven approach to derive dietary patterns as undertaken in the Generation R study and UK Pregnancies Better Eating and Physical Activity Trial (UPBEAT) studies (Flynn *et al.*, 2016), provides a practicable yet comprehensive assessment of the dietary behaviours of pregnant women which overcomes the limitations of single nutrient analysis (Hu, 2002).

Findings from the Southampton’s Women survey have recently demonstrated that the gestational period at which maternal nutrition is determined may influence the degree of adiposity in the child (Okubo *et al.*, 2014). This group reported that early dietary glycaemic load and glycaemic index assessed at 11 weeks’ gestation, following adjustment for potential confounders was positively associated with offspring fat mass at 4 and 6 years (n=906; fat mass SDs per 10-unit glycaemic index increase: p=0.02 at 4 years; p=0.01 at 6

years, fat mass SDs per 50-unit glycaemic load increase: $p < 0.001$ at 4 years, $p = 0.007$ at 6 years) in comparison to maternal dietary intake collected at 34 weeks' gestation.

Observational studies such as these have provided some credence to the hypothesis that maternal dietary manipulation could influence childhood adiposity, however evidence from RCTs in pregnancy are needed to establish causality.

Differentiating the effects of maternal obesity from maternal diet is obviously difficult to achieve in studies of pregnant women due to interaction between the two. It is therefore important that animal studies have shown proof of principle in this regard. Offspring of obese animals demonstrate similar phenotypes to the children of obese mothers (further details in *Section 1.4*) and these studies have provided evidence that diet alone e.g. high fat diet, can influence offspring obesity independent of maternal obesity (Samuelsson *et al.*, 2008; Parlee and MacDougald, 2014).

In summary pre-pregnancy obesity, excessive GWG, GDM and maternal diet may be important determinants of later childhood adiposity and are therefore considered to be modifiable factors for the prevention of childhood obesity *in-utero*.

1.3.11 Exposures in early infancy

In addition to exposure to an adverse *in-utero* environment, early postnatal life exposures have also been implicated in the development of childhood obesity, including mode and duration of feeding, childcare and sleep, all of which interact as illustrated in Figure 1.10, and could contribute to the modification of early-life growth velocities.

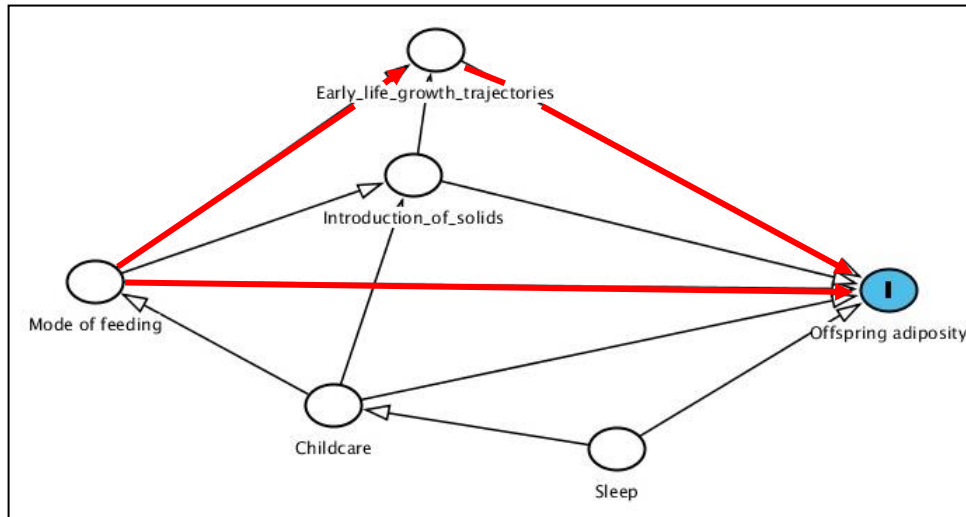


Figure 1.10: Interactions of early postnatal life exposures on offspring adiposity.

1.3.12 The relationship between mode of feeding and offspring adiposity was demonstrated as causal by a randomised controlled trial assessing lower protein vs. higher protein vs. breastfeeding and risk of later childhood obesity at 2 years of age as illustrated by a red arrow (Koletzko et al., 2009). Early life growth trajectories have been shown to influence the development of offspring adiposity in early adolescence, with the strength of association being robust, as illustrated by a red arrow (Ong et al., 2000; Tilling et al., 2011; Giles et al., 2015). However, the relationship between timing of introduction of solids, childcare and sleep with the development of offspring adiposity has primarily been derived from observational studies demonstrating inconsistent results (refer to Sections 1.3.12.3: Timing of introduction of solid foods, 1.2.13: Childcare and 1.3.14: Sleep. Postnatal feeding

1.3.12.1 Mode of infant feeding

Epidemiological studies have provided substantial evidence demonstrating that breastfed and formula-fed babies consistently follow different growth trajectories which may influence the risk of future obesity and other adverse health outcomes. The observation that obese women have reduced rates of initiation and duration of breastfeeding in comparison to normal weight women may therefore have implications for the development of obesity throughout the life course (Donath and Amir, 2008; Bever Babendure et al., 2015) .

Some studies have suggested that breastfeeding may influence infant regulation of appetite and satiety (Palou and Pico, 2009; Gridneva *et al.*, 2017) and breastmilk composition has been shown to be influenced by maternal age, diet, BMI and GDM (Nommsen *et al.*, 1991). The composition of formula milk has been continuously changing over the last decade. Formula milk, until recently has had increased energy and protein in comparison to breastmilk, which has been related to increased concentrations of branched chain amino acids (BCAA), non-esterified fatty acids (NEFAs), organic acids, acylcarnitines and phospholipids in the offspring; all indicated as potential biomarkers for obesity (Hellmuth *et al.*, 2016). The European Childhood Obesity Project, an RCT in which 1090 formula-fed infants were randomised to receive high or low protein content formula feed, has followed the children to 6 years of age. High protein formula feeding was associated with a 0.51 kg/cm² greater BMI and with a 2.43 increased risk of obesity at 6 years of age (Weber *et al.*, 2014) compared with formula feeding, suggesting potential long-term adverse outcomes in association with a high energy protein diet. A systematic review assessing 10 cohorts has also concluded that infants fed a lower protein formula had reduced weight and weight z-scores at 12 months, in comparison to those fed a high protein formula diet (Patro-Gołąb *et al.*, 2016b).

The Belarus Promotion of Breastfeeding Intervention Trial (PROBIT) was a RCT which sought to assess the effect of breastfeeding promotion on duration and exclusivity of breastfeeding (primary outcomes). Whilst the intervention had minor but significant effect on the duration of breast feeding, it was without effect on childhood obesity in early life (Kramer *et al.*, 2004; Owen *et al.*, 2005). However, the study was not designed to assess the effect of duration or intensity of breastfeeding on the risk of later development of obesity which was a secondary outcome (Patro-Gołąb *et al.*, 2016a). A recent meta-analysis including 105 studies determined that breastfeeding was associated with a reduced odds of developing obesity throughout the life course (OR 0.74, 95% CI 0.70 to 0.78), but the effect size significantly decreased following inclusion of only high quality studies (Horta *et*

al., 2015). One recent review also emphasises the promotion of breast feeding on the basis of protection against childhood obesity (Victoria et al, 2016).

1.3.12.2 Duration of infant breast feeding

The duration of infant breast feeding has been implicated with obesity risk in later life. Two systematic reviews have found a 20% reduction in obesity risk if the infant is exclusively breastfed in comparison with exclusively formula fed or mixed fed infants, suggesting a role of increased duration of exclusivity of breastfeeding in obesity protection (Arenz *et al.*, 2004; Owen *et al.*, 2005). Furthermore, an observational study of 2000 women assessing the role of different feeding modalities (formula vs. mixed vs. breast) found that if breastfeeding comprised of <80% of all feeds within the first 6 months; there was an associated excess infant weight gain between 6-12 months (Li *et al.*, 2008); a finding replicated in other populations including those exposed to GDM *in-utero* (Crume *et al.*, 2011).

1.3.12.3 Timing of introduction of solid food

Weaning is defined as a gradual transition from a milk diet (breast or bottle) to a solid food based diet which is associated with an increase in energy intake (Cohen *et al.*, 1994). Current WHO guidelines have recommended that solid food should be introduced from 6 months of age. In the UK, the average age of weaning is currently estimated at 4.6 months (Moore *et al.*, 2014).

Observational studies have demonstrated an association between early introduction of solids and increased adiposity in later childhood. A recent systematic review including 26 predominantly cohort studies reported that the introduction of solids <4 months of age was associated with an increased risk of developing obesity (Daniels *et al.*, 2015). However the authors concluded that residual confounding could not be discounted. The review was unable to establish whether introduction of solids in older children, at 4-6 months or 6

months resulted in increased obesity risk due to limited evidence and methodological limitations (Daniels *et al.*, 2015). Within an observational cohort, the delayed introduction of solid foods was associated with a reduced odds of childhood obesity at 10 years of age, independent of parental and child confounding (n=307; AOR 0.903 (95% CI 0.84 to 0.97); p=0.005) (Seach *et al.*, 2010). This may be due to modification of early-life growth trajectories as a study in the UK birth cohort showed that early introduction of solids in infants formula fed predicted weight gain between birth to 3 years; mediated through increased energy intake (Ong *et al.*, 2006).

Formula feeding is generally associated with the infant being weaned at a younger age, together with increased consumption of calories and saturated fat. Weaning dietary composition in infancy has been shown to reflect maternal dietary composition (Robinson *et al.*, 2015a). The mode of early infant feeding has also been shown to influence dietary composition of solids consumed in early infancy. For example, infants breastfed were shown to consume a healthier diet in a study from Holland of 2000 7 year old children; the study identified that those who were breastfed for at least 4 months were more likely to consume more fruit and vegetables, less soft drinks and fried food in comparison to those children who had been formula fed (Scholtens *et al.*, 2008). These studies suggest that breastfeeding, together with later, rather than earlier introduction of solid foods may be protective against childhood obesity.

1.3.12.4 The influence of *in-utero* exposures on postnatal feeding

Whilst the discussion above relates to exposure to *in-utero* or early life risk factors leading to childhood obesity, there is some evidence for interaction between the two. Infants may therefore experience a cumulatively increased risk of later obesity. A recent longitudinal cohort study carried out in 2014 found that maternal pre-pregnancy obesity significantly influenced early infant feeding practices. Mothers with a higher BMI were less likely to

exclusively breastfeed and more likely to introduce solid foods earlier than those of a normal BMI (Kitsantas *et al.*, 2016). Given the number of the factors associated with the development of childhood obesity, it is very important that potential *in-utero* confounders are accounted for including parental BMI, smoking, diet, activity levels and socioeconomic status in establishing potential associations of the maternal *in-utero* and early life environment and offspring adiposity.

1.3.13 *Childcare*

Many infants attend nursery or childcare facilities where meals and snacks are provided, which may promote early attitudes and beliefs regarding food. A study in 12,400 infants, found that infants cared for within an informal childcare setting were more likely to be overweight (adjusted risk ratio (ARR) 1.15; 95% CI 1.04-1.27) in comparison to those cared for by their parents (Pearce *et al.*, 2010). Furthermore, stratification by socioeconomic status found that this risk increased amongst affluent families. This was replicated in a Canadian observational study of 1649 infants, in which infants who attended childcare in comparison to parental care were more likely to be overweight and obese throughout a ten-year follow-up period (Geoffroy *et al.*, 2013). These findings add to the evidence that increased risk of obesity may be mediated at least in part through poor diet composition within early life.

1.3.14 *Sleep*

Sleep variation, both in duration and pattern, may also contribute to increased risk of childhood obesity. A longitudinal prospective cohort study found that from infancy to age 7, children with the lowest sleep scores (i.e. poor sleep) had a higher BMI, total and truncal fat mass than children with high sleep scores (Taveras *et al.*, 2014). Conversely, a Danish study found no association between sleep duration and measures of adiposity in infants aged up to 3 years (Klingenberg *et al.*, 2013). At present, there is limited knowledge as to

whether reduced early sleep duration is associated with the development of later obesity and insulin resistance in older children and observational studies, as assessing sleep as a potential exposure is subjective and complicated by recall bias.

1.4 Mechanisms implicated in the relationship between maternal obesity and childhood adiposity

The following discussion addresses the many different biological pathways which have been implicated in the relationship between maternal obesity and later determinants of obesity in the child. These include adverse influences of maternal overnutrition on the developing embryo, overproduction of fetal insulin, maternal inflammatory and metabolic imbalance leading to adaptive responses in the fetal hypothalamus and adipose tissue (Waterland and Garza, 1999; Mamun *et al.*, 2014). An understanding of the mechanisms associated with exposure to maternal obesity *in-utero* will enable the development of targeted interventions, within this high-risk population to address potential causative relationships and strategies to prevent childhood obesity.

1.4.1 Background

Mechanisms implicated with developmental programming have been summarised in Figure 1.11.

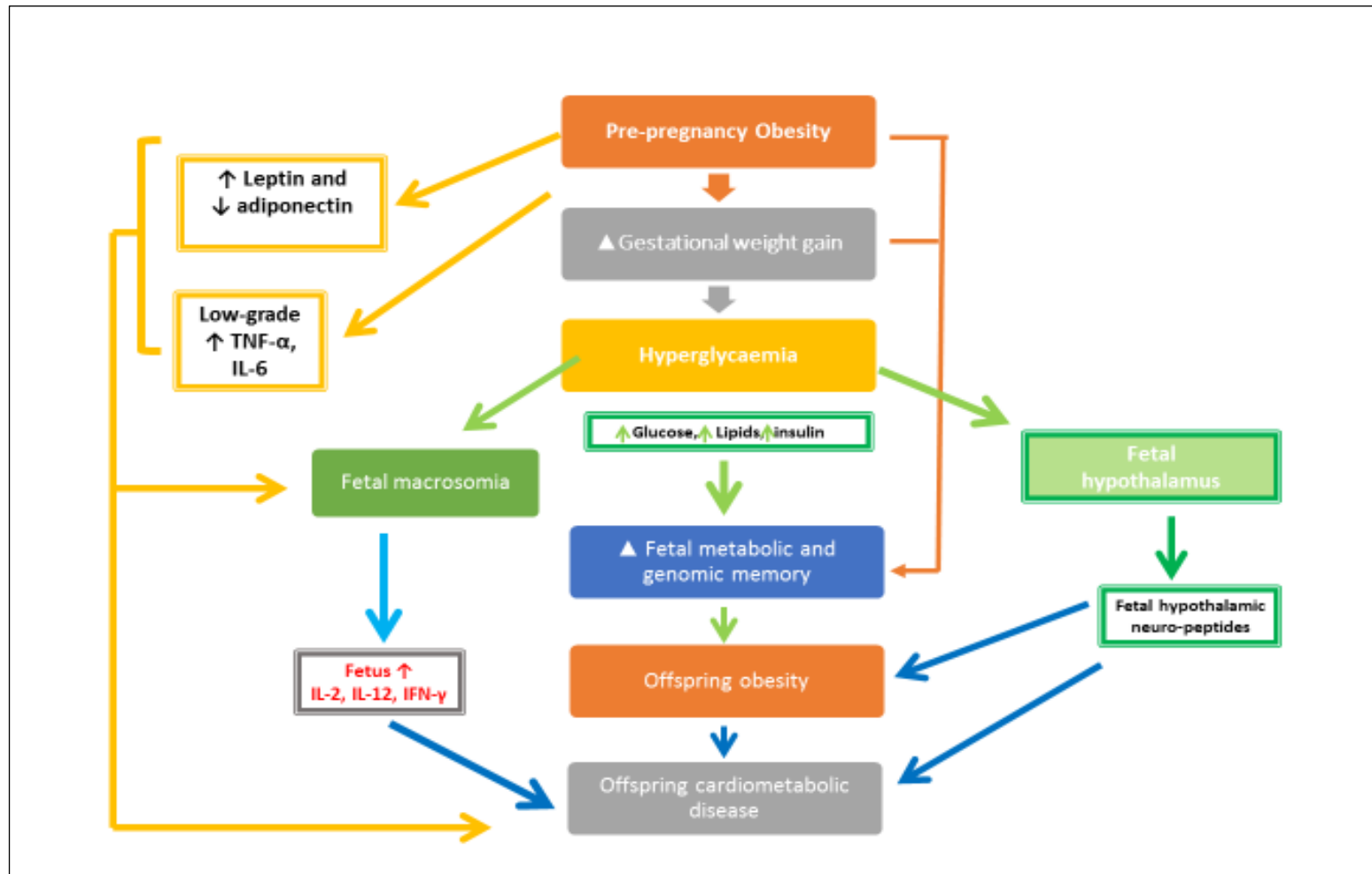


Figure 1.11: Biological mechanisms commonly implicated in the relationship between exposure to maternal obesity *in-utero* and offspring obesity.

1.4.2 Oocyte quality and subsequent embryogenesis

Obesity may have biological influences in the gamete at the earliest stages of life. Recent data from 218 oocytes from 29 women attending an in-vitro fertilisation clinic showed that increasing maternal BMI at conception was associated with phenotypic changes in the early embryo (Leary *et al.*, 2015). In another report, obese women (n=32) attending an infertility clinic had higher than normal follicular fluid insulin and lipids, associated with poor quality of the oocytes (Robker *et al.*, 2009). Leary *et al.*, also showed reduced glucose consumption, increased endogenous triglycerides and abnormalities in amino acid metabolism in oocytes and maternal blastocysts from obese women; suggesting metabolic impairment (Leary *et al.*, 2015). Experimental studies in diabetic and high fat fed mice models have demonstrated oocyte spindle defects, increased rates of follicular apoptosis and mitochondrial abnormalities (Seli *et al.*, 2014) as well as increased oocyte lipotoxicity, with mitochondrial dysfunction (Dumollard *et al.*, 2007; Wu *et al.*, 2010). Our laboratory has also reported mitochondrial abnormalities in oocytes and early blastocysts from obese mice in comparison to lean dams which were associated with increased oxidative stress (Igosheva *et al.*, 2010). These influences of maternal obesity on the oocyte and early embryo may have lasting consequences for the health of the offspring, as has been demonstrated with maternal under nutritional states in the rat (Kwong *et al.*, 2000).

1.4.3 Epigenetic modifications

Growing evidence suggests that environmental factors including modifications of the diet in early life can alter the fetal or early postnatal epigenome. The hypothesis suggests that persistent modifications of the epigenome determine offspring gene expression whilst preserving nucleotide sequence and can subsequently influence metabolism in later life. Figure 1.12 summarises the process of the environmental exposure influencing the

genotype and subsequent phenotype. Associated mechanisms related to this change in gene expression could be a result of histone modification or DNA methylation.

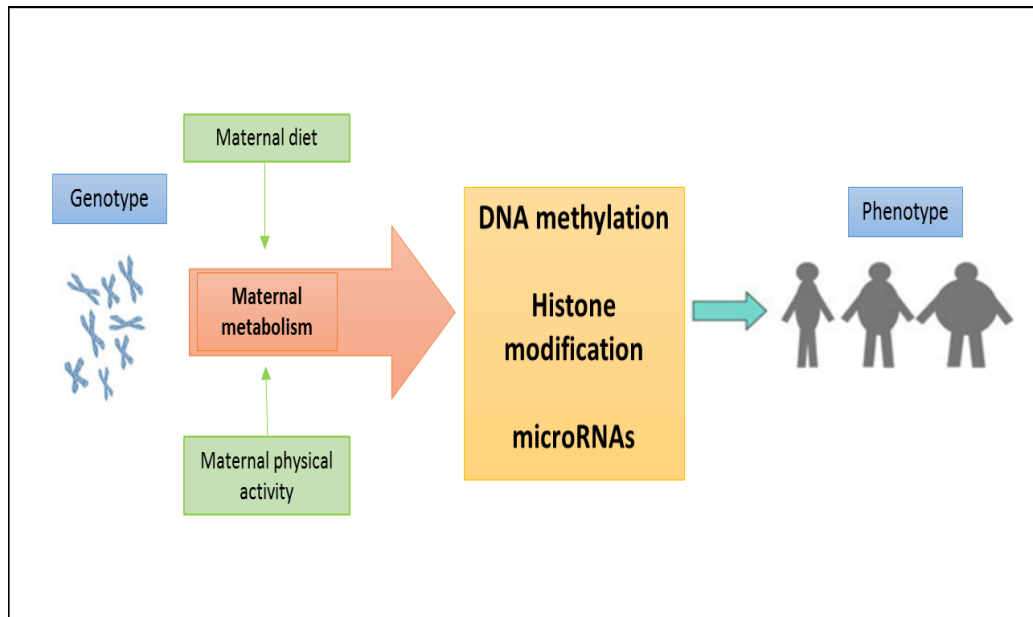


Figure 1.12: The influence of environmental exposure on the genotype and subsequent phenotype.

Modifications in DNA methylation at CpG sites and post-transcriptional modifications on histones result in alterations in the chromatin profile. The combination of these alterations result in modification of the phenotype. Adapted from Cordero P, Li J, Temple JL et al. Epigenetic Mechanisms of Maternal Obesity Effects on the Descendants. Parental Obesity: Intergenerational Programming and Consequences; Physiology in Health and Disease. 2016;1:355-368 (Cordero et al., 2016).

Several independent reports in different animal models of varying nutritional status highlight how changes in maternal diet associated with persistent metabolic dysfunction in the offspring, are accompanied with epigenetic changes in key genes involved in appetite control and metabolism (Cole *et al.*, 1993; Lillycrop *et al.*, 2005; Lillycrop *et al.*, 2007; Lillycrop and Burdge, 2015). Of the few studies undertaken in mother-child pairs, one report by Godfrey *et al.* has shown that alterations in epigenetic biomarkers can be predictive of later adiposity risk; for example, methylation of a single CpG site in the promoter region of the nuclear receptor Retinoid X Receptor- α (RXRA) in cord leucocytes was associated with the development of childhood adiposity (mean age 6.5 years) in two independent cohorts (Godfrey *et al.*, 2011). Higher methylation of RXRA, was associated with reduced maternal carbohydrate consumption previously associated with greater

offspring adiposity (Godfrey *et al.*, 2011). This independent finding provides evidence of a greater effect size estimate in relation to offspring adiposity than that observed with neonatal birthweight or maternal body composition; suggesting that epigenetic changes as a result of the *in-utero* environment may provide an improved assessment of later childhood obesity. A recent report using a epigenome-wide approach (Illumina Infinium® HumanMethylation 450 K BeadChip) in the ALSPAC cohort (1018 maternal-offspring pairs), has provided some of the strongest evidence to date in support of a change in DNA methylation status (as measured in cord leucocytes) associated with relationships between maternal obesity and increased offspring adiposity at mean ages of 9.9 and 15.5 years, in comparison to offspring from normal weight women (Sharp *et al.*, 2015).

Persistent changes in the epigenome offer a final pathway linking early life exposures such as maternal obesity with offspring health outcomes. Other interactions between environment and gene in fetal development may transiently influence gene expression causing lasting perturbations in organ growth and development (Godfrey *et al.*, 2007; Sullivan and Grove, 2010; Sovio *et al.*, 2011; Hanson *et al.*, 2014; Cordero *et al.*, 2016) .

1.4.4 Hypothalamic changes in relation to the epigenome

In animal models there is a well characterised leptin ‘surge’ in early postnatal life which plays a critical role in normal neuronal development of the hypothalamus (Bouret, 2010). Maternal obesity is associated an increased magnitude of the leptin surge in rodent offspring (Kirk *et al.*, 2009). Although the origin is uncertain, there is no evidence of an association between maternal milk leptin and offspring serum leptin in rodents (Bautista *et al.*, 2008; Kirk *et al.*, 2009), whereas neonatal leptin is associated with increased expression of the Ob gene (the leptin gene) in offspring adipose tissue (Kirk *et al.*, 2009). Samuelsson *et al* has also reported that exogenous leptin administration within normal pups is associated to the development of obesity and cardiovascular dysfunction in adult life

(Samuelsson *et al.*, 2013) indicating a potential associative role in long term adverse cardiovascular health and hyperphagia.

1.4.5 Potential maternal influences as a cause of epigenetic modifications

Maternal obesity is associated with changes in maternal metabolism which may be causative of offspring epigenetic modifications, persistent changes in tissue structure and function, associated with later development of an obese phenotype. In this thesis, metabolite exposures have been assessed by metabolomics. Metabolomic profiles signify the cellular response and represent a key link between the genotype and phenotype. This technique has become widely used to identify metabolite pathways modified by disease or adverse exposures. It is now a well-established methodology providing a “top-down” approach that enables exploration of the downstream products of modifications to gene transcription (Figure 1.13).

Metabolomics provides a novel methodological approach to gain insight into the mechanistic pathways associated with DoHaD (Rauschert *et al.*, 2014; Hivert *et al.*, 2015). To date, very few studies have adopted this approach when assessing the *in-utero* origins of obesity.

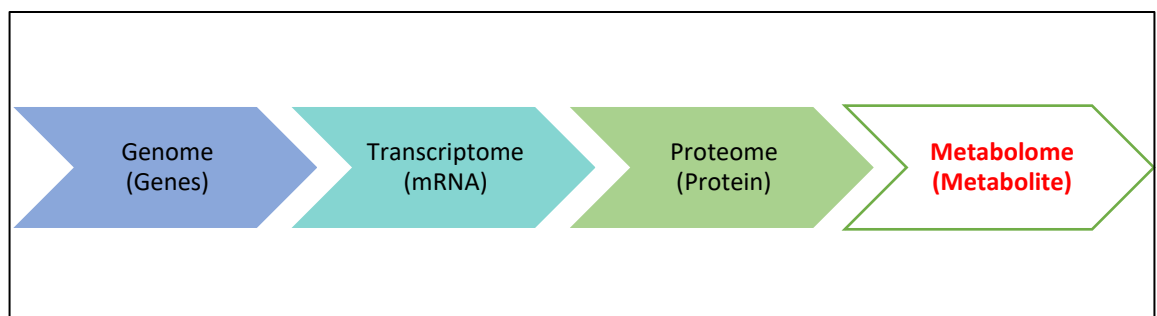


Figure 1.13: The integration of the "Omic" classification and their associated downstream products.

Metabolomics is the downstream product of the genome allowing the study of the metabolite profile within a system under given conditions.

In relation to maternal obesity, two studies have, to date assessed the maternal metabolomic profile in relation to neonatal body composition (Scholtens *et al.*, 2014; Scholtens *et al.*, 2016). Network analysis from 400 maternal samples from the HAPO study has demonstrated clusters of fatty acids and carnitine esters that were related to maternal fasting glucose at 20-29 weeks' gestation, and subsequently with neonatal adiposity. Neonatal adiposity was also associated with the concentrations of ketone bodies, amino acids and fatty acids measured in samples collected at one hour post glucose load at the time of an oral glucose tolerance test (OGTT) (Scholtens *et al.*, 2016). This approach, which has proved invaluable in understanding the metabolic profile in type 2 diabetes and cardiovascular, is open to investigation in contemporary cohorts of obese mothers and their children.

To date, few studies have addressed whether the cord blood metabolome at birth, as a proxy of fetal exposures, could provide insight into mechanisms of programming by early life maternal exposures. One report has examined the metabolome in low birthweight (n=20) and normal birthweight (n=30) neonates, demonstrating differences in the clustering of amino acids and lipids. The metabolic profile associated with low birthweight demonstrated similar patterns to type 2 diabetic adults (Isganaitis *et al.*, 2015). A recent study assessed the association of the maternal urinary metabolome with that of the offspring's cord blood, stratified by pre-pregnancy BMI (normal, overweight and obese) in 321 maternal-offspring pairs. There was no significant differences in the cord blood metabolic profiles of offspring stratified by maternal BMI group; although infants born to obese mothers had a higher birth weight and lower Apgar Scores (Desert *et al.*, 2015).

1.4.6 Maternal stress and inflammation

See also *Section 1.4.4*. Obesity is associated with the development of chronic systemic low grade inflammation in adipose tissue, hypothalamus, liver and muscle (Ingvorsen *et al.*,

2015) and there is a suggestion that inflammation *in-utero* predisposes the offspring to metabolic compromise from birth (Catalano *et al.*, 2009) (Figure 10). Low grade maternal inflammation has been shown to lead to both short and long term epigenetic modification of fetal genes involved in regulation of the central hypothalamic pituitary axis (HPA) pathway (Moisiadis and Matthews, 2014). Glucocorticoids readily cross the placenta and enter the fetal brain (McCabe *et al.*, 2001) and it is proposed that downregulation of the fetal HPA axis permanently influences myelination and neurogenesis of the fetal brain and may influence hypersensitivity within the peripheral organs responsive to the HPA pathway (Moisiadis and Matthews, 2014). Sheep models have demonstrated that excess glucocorticoid exerts a persisting influence on the adult offspring including increased blood pressure, glucose and insulin levels (Gatford *et al.*, 2000).

A growing body of evidence show that changes in the inflammatory profile are predictive of later cardiometabolic disease in the non-pregnant population (Dandona *et al.*, 2004). Whether this pathway may be of relevance to maternal obesity and influences the developing fetus is not known, as studies have demonstrated inconsistent associations (Reynolds, 2013). Whilst there are reports of raised maternal glucocorticoids in obese rodents (Zambrano and Nathanielsz, 2013), there is no evidence of increased glucocorticoids in obese pregnant women (Reynolds, 2013).

1.4.7 Maternal adipokines

Maternal inflammation is associated with increased circulating adipokines, both contributing to the insulin resistance pathophysiology. The principal adipokines implicated with maternal obesity and subsequent offspring obesity are leptin and adiponectin (Figure 1.11). As discussed previously in the *Section 1.4.4*, leptin has been implicated in the ‘re-wiring’ of the fetal hypothalamus, associated with offspring adiposity. Maternal adipokines play an important role in energy balance regulation and are related to adipose tissue mass.

Maternal leptin and fasting glucose concentrations are linearly associated with cord leptin concentrations, a proxy measure for neonatal fat mass (Geary *et al.*, 1999; Fairley *et al.*, 2015). In a prospective birth cohort including 599 children, cord blood leptin concentrations were associated with reduced birthweight, but increased weight gain within the first 6 months of life; suggesting potential modifications in appetite and satiety regulation within early life (Mantzoros *et al.*, 2009)

1.5 Defining neonatal and infant adiposity

As this thesis, has addressed “*in-utero* and early life origins of infant adiposity born to obese women”, methods of assessment of infant adiposity will be discussed in the section below.

Distribution of adiposity is defined based on its anatomical site. It is divided into two main locations; subcutaneous and visceral. Visceral adipose tissue is associated with features of hyperglycaemia and hyperinsulinemia (Ibrahim, 2010), whereas subcutaneous adiposity is thought to be highly sensitive to insulin and hence protective against cardiometabolic disease (Moran *et al.*, 2017). Measurements defining increased levels of adiposity in children are needed that are closely related to the accumulation of body fat, independent of overall body size, as a child’s body size modifies during the different phases of development.

1.5.1 Measures of general adiposity

General adiposity is defined as total body fat regardless of anatomical distribution.

1.5.1.1 Weight

Weight is a simple and easily collected measure that is reasonably correlated with total body fat within adult populations irrespective of sex. However, in children, weight is variable due to age, sex and height. Current WHO recommendations, adopted in clinical practice in the UK, suggest that plotting the child's height and weight on sex-specific growth trajectories provide an assessment of longitudinal growth including development of obesity over time (de Onis and Blössner, 2003).

1.5.1.2 Body mass index

BMI is defined as weight in kilograms divided by the square of height. At present, there is no universally agreed definition for childhood obesity and overweight due to a variety of methodologies and thresholds available (de Onis *et al.*, 2010). Using simple thresholds, as in the adult population, has limited use in children, as BMI varies significantly by gender and age groups (Berentzen *et al.*, 2016). This lack of continuity between childhood and adult measurements could lead to significant disparities when assessing obesity throughout the life course.

1.5.1.3 Growth reference curves

The WHO's development of growth charts for infants aged between 0-5 years has enabled longitudinal tracking of obesity within early childhood. These charts recommend the use of standardised z-scores for children from birth to age 5 years. The z-score expresses the anthropometric value as a number of standard deviations below or above the reference mean or median value. Prevalence based data are commonly reported using a cut-off values (<-2 and $>+2$ z-scores). However, it should be noted that the rationale behind the cut-off values are based on the statistical definition which assumes that 95% of values centred around the mean is defined as the "normal" range, which may not necessarily be an optimal

point in relation to clinical or prediction of adverse outcomes. Ideally, the criteria should be established based on their associations with the risk of adverse growth.

Overweight and obesity is defined as BMI >2 or >3 standard deviations above the WHO growth standard median respectively (WHO, 2006). Differences arise due to various thresholds for defining obesity, from different reference populations, making comparisons to infant anthropometric measures problematic. The WHO growth centiles were derived from an international sample from Brazil, Ghana, Norway, Oman and United states including 26,985 infants. These cohorts also provide longitudinal follow-up anthropometric measures of length and weight from birth to 24 months and a cross sectional survey of children aged between 1.5 years to 6 years of age (de Onis and Blössner, 2003; WHO, 2006).

An appreciation of other reference populations is essential when drawing comparisons from other studies using various definitions. The UK90 BMI reference centile curves are based on a sample of 32,222 measurements from 12 surveys, collected between 1978 to 1994, before the exponential rise in childhood obesity (Wright *et al.*, 2002). These provided reference data in children from birth to 23 years. Here childhood obesity is defined as $\geq 95^{\text{th}}$ centile for population monitoring and 98^{th} centile for clinical assessment (Wright *et al.*, 2002). The use of two different cut-offs for the same condition, provides difficulty in tracking obesity through the life course and results in inconsistencies in findings from a clinical and research settings.

The International Obesity Task Force provides growth centiles curves for children aged between 2-18 years derived from multiple international cohorts ($n=192,727$), including Brazil, UK, Hong Kong, Netherlands, Singapore and United States (Zimmermann *et al.*,

2004). Age specific cut-offs points were extrapolated from adult BMI cut-offs for overweight and obese, which are widely used.

The thresholds, as defined by the WHO Global Database on Child Growth and Malnutrition, are used to define infant overweight and obesity for the purpose of this thesis (de Onis and Blössner, 2003), as these are applicable irrespective of mode of early life feeding, ethnicity and socioeconomic deprivation. Table 1.2 summarises the WHO thresholds which will be used for this thesis.

Variables	Z-Score	Definition
Z-score Thresholds will be used for WLZ and WAZ standard deviation scores	<-3 Standard Deviations	Severe infant undernutrition
	<-2 Standard Deviations	Infant under-weight for defined parameters
	>+2 Standard Deviations	Infant over-weight for defined parameters
	>+3 Standard Deviations	Infant obesity for defined parameters
BMI (kg/m ²)	≤ 2 nd centile	Underweight
	>2 nd to <85 th centile	Healthy weight
	≥85 th centile	Overweight
	≥ 95 th centile	Obesity

Table 1.2: WHO thresholds used for the definition of infant overweight and obesity at 6 months of age.

Abbreviations: WAZ- weight for age z-score; WLZ- weight for length z-score.

As previously mentioned, BMI is an inaccurate estimate of body fat mass in children, therefore accurate estimation requires an understanding of infant body composition as well as complex methodologies used in its assessment.

1.5.2 Methods of measuring infant body composition

To address the hypothesis that the *in-utero* environment associated with maternal obesity influences neonatal and infant fat deposition, accurate and practical assessment of body composition was necessary for the work described in this thesis.

Body composition is traditionally classified as two component models; where assessment of fat mass and fat free mass is made. The use of a multi-compartment model further divides fat free mass into protein, water, mineral and bone. These distinctions are essential as when BMI is measured, it is not known whether this increase is due to increased fat mass or decreased fat free mass. Distinguishing between fat mass or fat free mass is important as total body water and mineral content are higher in obese children, and the proportion of protein is reduced in comparison to non-obese children (Haroun *et al.*, 2004).

1.5.2.1 Isotope dilution (hydrometry)

Estimated fat free mass can be derived from measurement of total body water by deuterium dilution. Here, a small volume of water labelled with deuterium is given and assessed using an isotope ratio mass spectrometry once ‘enrichment of the total body pool’ has been reached (Wells and Fewtrell, 2006). For this methodology, fat free mass is estimated on an assumed value of hydration from published reference values. This method is advantageous as it provides an accurate longitudinal assessment of adiposity throughout the life course. Although this methodology is simple, inexpensive to undertake and reliable, it has limited use in infants due to potential dose spillage, time to achieve enrichment of the total body pool and difficult in collection of samples (Traver *et al.*, 2009).

1.5.2.2 Air displacement plethysmography (PEAPOD)

This method assesses body volume by detecting subtle differences in air pressures between the infant (test chamber) and reference chamber that has controlled, pre-defined air pressure. Fat mass, fat free mass and percent of body fat is calculated using body mass, volume, density of fat, age specific coefficients (Demerath and Fields, 2014). Although a relatively new method, it has been validated for use in both neonates and infants and is also found to be reliable for repeated measures in early life (Fields and Allison, 2012). This

methodology has been undertaken at birth and 2 months of age in the Cork Baseline Birth Cohort study (O'Donovan *et al.*, 2015), where detailed measures of fat mass were able to predict childhood BMI accurately in 922 infants (Hawkes *et al.*, 2016). This method has advantages over DEXA as it does not involve radiation exposure therefore enabling serial and comparable measurements. Although, this method for assessing adiposity was made available in the UPBEAT study to measure neonatal adiposity, it had limited acceptance with mothers at the time of delivery and was practicably difficult to obtain as the neonates were discharged from hospital within 24 hours following delivery.

1.5.2.3 Dual energy X-ray absorptiometry

This methodology involves the use of X-ray with varying photon energies to determine body composition. The use of DEXA has been found to be more accurate than the use of SFTs, however it may result in a cumulative radiation exposure (Wells *et al.*, 2012). Whilst DEXA can be used in swaddled neonates, parental consent is frequently disallowed because of exposure to X-rays. In young infants and children under the age of 5 years, DEXA is also seldom used due to child movement.

1.5.2.4 Bioelectrical impedance

Bioelectrical impedance can also be used to assess body fat mass. The principal of bioelectrical impedance measurement of body fat relies on the fact that lean body mass is a good conductor of electricity because of a high fluid and electrolyte content, whereas adipose tissue and bone are resistant. Bioelectrical impedance assesses the direct relationship between the electrical conductivity and concentration of ions (lean body mass), together with the indirect relationship between the ion concentration and resistance to conductivity (fat mass) (Dehghan and Merchant, 2008). Assumptions are made including the validity of the derived prediction equations for the population assessed and that the intracellular to extracellular ratio remains constant. Bioelectrical impedance is validated

against known reference methods including DEXA in children aged 4-18 years, but has provided inconsistent results in neonates and infants (Ellis *et al.*, 1999; Sesmero *et al.*, 2005). Validation studies have found that bioelectrical impedance underestimates body fat in lean infants (aged 2.4 years) and overestimates it in obese infants (Horan *et al.*, 2015), therefore this method was not chosen for use in the UPBEAT infants at 6 months of age.

1.5.2.5 Skinfold measurements

Skinfold thickness measurements using validated callipers have provided a reliable estimate of subcutaneous fat for many years (Freedman *et al.*, 2009). Under experimental conditions, these measurements provide an accurate estimate of total body fat in children (Freedman *et al.*, 2009). Although training is required to ensure accuracy, these measurements are inexpensive. Childhood skinfold measurements in cohort studies are predictive of later cardiometabolic health in comparison to the use of other measurements including waist circumference (Juonala *et al.*, 2011); suggesting that these measurements may provide a better reflection of adiposity. Measurement of skinfolds are prone to measurement error if incorrectly undertaken, which may limit translatability (Mueller and Malina, 1987; WHO and de Onis, 2006). Within infants, measurements commonly undertaken include subscapular SFT, a measure of central adiposity and triceps SFT, a measure of peripheral adiposity. In light of several studies reporting associations between early life exposures and later childhood adiposity as measured by SFTs, this method was chosen for the present study as it is reliable, inexpensive and feasible in young infants.

1.5.2.5.1 Estimated total fat mass

SFTs can be used to estimate total fat mass using validated, formulated equations (Slaughter *et al.*, 1988). Using this method, estimate of total body fat are highly correlated to those obtained by DEXA (Slaughter *et al.*, 1988). The sex-specific equations reported by

Slaughter et al proposed the use of 2 SFTs to predict fat mass, which are used within this thesis.

1.5.2.5.2 Subscapular: Triceps ratio

This measure reflects a central skinfold measure to a peripheral measure, which have been shown to moderately predict dyslipidaemias (area under receiver operator curve ≥ 0.7), with the exception of low HDL, in 905 Mexican children at 6 years of age (García *et al.*, 2011). This measurement, may therefore provide an accurate assessment of central adiposity and associated adverse cardiometabolic traits in response to exposure to an adverse *in-utero* environment.

1.5.2.6 Abdominal, waist and arm circumferences

These circumferences are easily obtained within a clinical or research setting. The three measurements provide an indirect measurement of visceral fat mass and in adults are associated with an increased risk of type 2 diabetes, hyperlipidaemia and cardiovascular disease. However, in neonates this measurement is largely variable as the reliability is influenced with feeding. Despite these limitations, these circumferences together with BMI and skinfold measurements throughout childhood may provide insight into the deposition of central adiposity into adulthood (Jago *et al.*, 2013).

1.6 Caveats and drawbacks of the developmental programming literature

1.6.1 Observational studies

The fundamentals of the ‘developmental origins of adult disease’ hypothesis were derived from observational data, with focus on *in-utero* exposure of risk factors during early life periods of development plasticity on a given offspring outcome. Population based birth cohorts have been paramount in underpinning our understanding of early life determinants

of later disease; in particular, the relationship between birthweight with adulthood cardiovascular disease (Barker *et al.*, 1989; Barker, 2002). More recently, investigations of a myriad of maternal variables including diet, behavioural factors, biomarkers and the metabolome, as well as the epigenome in the infant and child has facilitated the investigation of biological pathways by which environmental exposures may convey disease risk in adulthood (Godfrey *et al.*, 2016). Distinguishing causality from association, whilst difficult, is essential to identify key early life modifiable causes of infant adiposity, and thereby direct interventions to key targets.

Observational studies describe a natural process, in contrast to a controlled experiment. The long interval between measurement of exposures (both maternal and early infancy) and outcome data (childhood, adulthood) can be problematic if there is limited availability of longitudinal outcome, exposure and confounder data. Confounding factors or biases can influence the association within longitudinal studies, and thereby the outcome of interest. Statistical adjustment for confounders in multivariate modelling techniques may not fully correct for the effects of social interaction for example, and unadjusted confounding is a common cause of inconsistency between results from cohorts and RCTs (Mathews *et al* 1999, Poston *et al* 2006). Another consequence of these long intervals is the questionable relevance of the observations to contemporary pregnant populations.

Although evidence has accumulated in support of the DoHaD, some critics have argued there are alternative explanations for the observed associations. These include confounding by genetic factors and sociodemographic characteristics. It has been suggested, for example, that variants of the Insulin Receptor Substrate-2 gene may account for the observed association between low birthweight and vascular disease, as this is a pleiotropic gene which theoretically could result in two phenotypes, one in the fetus (low birthweight) and the other in the adult (type 2 diabetes and cardiovascular disease) (Hattersley and Tooke, 1999). Similarly, two others variants, an ACY5 allele and ADRB1 gene have been

implicated in both offspring birth weight and adult-onset hypertension and type 2 diabetes (Li *et al.*, 2001). The social environment may also play a causal role. Some, but not all studies have adjusted for baseline maternal socioeconomic deprivation as assessed by the Index of Multiple Deprivation; at best a crude proxy of socioeconomic status (Caspi *et al.*, 2016). Furthermore in observational epidemiology causal inference is also limited due to selection bias and reverse causation (Gaillard, 2015).

Robust statistical methods have been developed, considering the limitations of observational epidemiology to enable further insight into the role of confounding factors in relation to maternal exposures and offspring outcomes and to further strengthen causal inference. These include maternal and paternal offspring comparisons, MR and the use of instrumental variables robustly associated with the exposure, thereby controlling for confounding and measurement error (Richmond *et al.*, 2014). Sibling comparison studies offer an innovative approach to enable determination of the causal relationship between maternal exposures and offspring outcomes; Lawlor *et al* found that increasing maternal weight gain was associated with increased offspring BMI at 18 years in only overweight and obese mothers (n=146,894 individuals from 136,050 families) after controlling for familial genetic and environmental influences (Lawlor *et al.*, 2011a). By using a within and between sibling association study design, the environmental and social confounders are effectively controlled for, thereby providing convincing evidence of a causal relationship of the intrauterine mechanisms of weight gain and later obesity. However, this methodology is also not without its limitations. Sibling comparison studies are formulated on the assumption that the effect of each participant's exposure to a risk factor does not influence other unexposed outcomes (Rubin, 2006).

MR is a statistical procedure based on the assumption that candidate genetic variants which are known to reflect biological effects are used to assess the effect of a potential

environmental exposure on a disease risk (Smith and Ebrahim, 2003). Genetic variants are used as are thought not to be influenced by behavioural, psychological or social circumstances (Smith, 2006). The use of MR, through the use of sophisticated statistical methodology has provided support for the developmental overnutrition hypothesis as the association may not be present in the preconception period (Lawlor *et al.*, 2008). Although MR enables establishment of causality with a certain degree of certainty, it requires a large sample size and the methodology is not suitable for genetic variants having a direct pleiotropic effect on both exposure and outcome of interest (Smith and Ebrahim, 2003).

1.6.2 Use of animal models

Observational cohorts have provided a strong association between a sub-optimal *in-utero* environment and the development of adverse offspring outcomes (Patel *et al.*, 2015). Animal models have been the primary tool to understand mechanisms implicated with the developmental programming hypothesis (Patel *et al.*, 2015).

Replication in rodent models, where diet can be tightly controlled further enables the examination of the underlying physiological and biochemical mechanisms behind the nutritional programming of offspring disease; further aiding identification of the mechanisms and critical windows of intervention (Litten-Brown *et al.*, 2010; Lillycrop and Burdge, 2015). However, it is critical to appreciate the differences in the stages of development between animals and man, to infer generalisability to humans. Rodents are altricial species, born with an underdeveloped endocrine system and brain, and undergo significant maturation of their organs during the weaning period, reflecting the third trimester in human pregnancies. Non-human primates are the most appropriate model, but are limited due to their long lifespan, resulting cost and ethical considerations. Many researchers choose rodents because of the shorter lifespan and lower costs. Sheep have a

similar rate of pre and postnatal growth to humans and produce fewer offspring than rodents, comparable to humans.

Although it is fully appreciated that there are many biological and species differences, response to interventions often provide valuable insight into the mechanisms implicated with improvement in human outcomes (Litten-Brown *et al.*, 2010). For example, increased physical activity during rodent obese pregnancies can potentially be used to determine the adult offspring response in humans. Animal models have been used to determine the effectiveness of interventions especially dietary and physical activity due to the ease of enforcing exercise and controlling diet. Interventions within the preconception period and during lactation have demonstrated an improved glucose homeostasis and adipocyte deposition in adulthood (Zambrano *et al.*, 2010). These studies provide a convincing basis to guide future human translational studies by allowing investigation of interactions between genetic and environmental factors. However, further investigation is required within animal models to determine the effective timing and intensity of such interventions in humans.

1.6.3 Intervention studies

The gold standard for the estimation of the causal effects implicated with lifestyle interventions on offspring outcomes is prospective RCTs. By undergoing randomisation, it is assumed that potential confounders in association with exposure and outcomes are avoided. Several RCTs have been extended to follow up of the offspring in mid-childhood. Intervention studies to date in obese women have focused on the prevention of excessive GWG or mild dysglycaemia and insulin resistance. The primary strategies have included advice to change dietary and physical activity through modification of lifestyle behaviours, and more recently the use of pharmacological intervention, for example the use of metformin.

Two recent notable follow up studies of a well conducted observational study and RCT have demonstrated that maternal fasting glucose during pregnancy is not associated with the development of childhood obesity at 5 to 7 years (n=1320). In the first study, Thaware *et al* followed-up participants of the HAPO study, a multicentre observational study undertaken to assess the associations of gestational hyperglycaemia with maternal and fetal outcomes (n=25,505) (Metzger *et al.*, 2008). In children of participants enrolled in a multicentre RCT to assess the treatment of mild GDM (n=958; control arm- normal antenatal care, intervention arm- dietary intervention, self-monitoring of blood glucose and/or insulin therapy (Landon *et al.*, 2009), were followed up at 5-10 years and treatment of mild hyperglycaemia was found not to be associated with a reduction in childhood obesity and metabolic dysfunction at 5-10 years (Landon *et al.*, 2015). However, follow up was unplanned and incomplete (n=500). Further sub-group analysis revealed that treatment of mild GDM was associated with a significantly lower childhood obesity in those infants with the greatest adiposity at birth (Landon *et al.*, 2015). These reports apparently challenge the role of maternal hyperglycaemia in the developmental programming of childhood obesity (Thaware *et al.*, 2015). Without an intermediate point in early infancy, an interaction between fetal and postnatal exposures cannot be assumed as components of postnatal growth, for example mode of infant feeding may confound the observed relationship (Castillo-Laura *et al.*, 2015). Both analyses adjusted for many prenatal confounders but neither accounted for growth and environmental factors including breastfeeding and diet in early infancy (Harder *et al.*, 2005; Ong and Loos, 2006).

The EMPOWaR study, a randomised double-blinded placebo trial, recruited obese pregnant women at 12 to 16 weeks' gestation with a normal glucose tolerance test, to receive metformin throughout pregnancy (Chiswick *et al.*, 2015). There were no differences in birthweight or in neonatal or maternal anthropometry. The study interestingly demonstrated its pharmacodynamic effects of metformin, including reduction

in fasting glucose, insulin and reduction in inflammatory markers (C Reactive protein (CRP) and interleukin 6 (IL-6)), all of which have been implicated with the developmental origins of adverse health and disease. Better pregnancy outcomes for the offspring have been reported in GDM diagnosed mothers with reduced central or visceral adiposity, due to metformin treatment (Rowan *et al.*, 2011), as well as improved fetal outcomes compared to those treated with insulin. The long-term follow of these pharmacological studies is required to determine the effects of metformin and whether this may be beneficial or not for offspring health.

In 2014, the LIMIT RCT in 2212 overweight and obese pregnant women published its findings. This was one of the first notable studies, of adequate sample size and study design which randomised overweight or obese women (n=2212) to lifestyle advice or standard antenatal care, with the primary aim to reduce delivery of a LGA infant (Dodd *et al.*, 2014b). Although the trial's primary outcome was not reached, the study found fewer infants who were born greater than 4kg in the intervention arm (15% vs. 19%; p=0.04) in comparison to standard care. Ongoing follow-up has been conducted and are yet to be reported. The low glycaemic index diet in pregnancy (ROLO study) to prevent macrosomia assessed the influence of a behavioural lifestyle intervention to prevent recurrence of a macrosomic delivery in 800 women (mean BMI 26.8 kg/m²) without a diagnosis of diabetes (Walsh *et al.*, 2012). Similar to the LIMIT study, the ROLO study found no difference in neonatal birthweight, but found significant reductions in maternal GWG (intervention 12.2 kg versus control 13.7 kg; p=0.01) and reduced glucose intolerance (intervention 21% vs. control 28%; p=0.02) (Walsh *et al.*, 2012). However, the lifestyle intervention, or its subsequent changes including GWG were not associated with changes in infant body composition or growth at 6 months of age (Horan *et al.*, 2016). Despite recent efforts, a Cochrane review of the available evidence from randomised trials (49 RCTs; n=11,444 women) suggests that although modification of maternal dietary intake

can occur, it has limited success in reducing GWG or GDM. Furthermore, the intervention studies included have not been successful in improving neonatal outcomes, including excessive fetal growth and caesarean sections (Bain *et al.*, 2015; Muktabhant *et al.*, 2015), but lacked long-term follow-up of offspring.

As discussed in *Section 1.3.8*, the incidence of GDM is strongly associated with maternal obesity, therefore it could be argued that interventions aimed at preventing GDM should be undertaken in high-risk populations. The Finnish Gestational Diabetes Prevention Study (RADIEL), assessed a behavioural intervention comprised of a physical activity and diet intervention in 540 pregnant women (Koivusalo *et al.*, 2016). Analysis was undertaken by intention to treat, however only 269 women were included within the final analysis. The incidence of GDM (intervention 13.9% vs. control 21.6%; 95% CI 0.40 to 0.98; $p=0.044$) and total GWG (mean difference -0.58kg; 95%CI -1.12 to -0.04; $p=0.037$) were found to be significantly lower in the intervention arm in comparison to the control arm (Koivusalo *et al.*, 2016), providing evidence that an individualised intervention has the potential to reduce the incidence of both GDM and GWG. However, replication within larger populations are required to determine whether these findings are causal as well as long-term follow-up of the offspring to determine a beneficial influence.

The recently undertaken RCT of a dietary and physical activity intervention, the UPBEAT trial in 1555 obese pregnant women, is the largest study powered for clinical outcomes, and recently published (Poston *et al.*, 2015). Women were randomised to standard antenatal care or an intervention delivered by health trainers over 8 weeks (weekly sessions). The intervention focused on improving glycaemic control through a low glycaemic index diet and increased physical activity. This intervention differed from previous studies, through development and delivery of the intervention. The intervention focused on approaches to achieve Specific, Measurable, Achievable, Relevant, Time Specific (SMART) goals as

well as advice on self-monitoring, social support and problem solving to barriers of behaviour change (Poston *et al.*, 2013). Women were advised on reducing saturated fat intake and glycaemic load as well as increasing time spent doing low/moderate physical activity. This study did not meet its primary endpoints of reducing maternal GDM or delivery of a LGA infant. However, significant changes were observed in maternal antenatal diet, physical activity and measures of maternal body composition throughout pregnancy. These included significant reductions in total energy intake (MJ/day), saturated fat (% energy) and glycaemic load per day and increased physical activity as assessed by the metabolic equivalent of task (MET) from 15⁺⁰-18⁺⁶ to 26⁺⁰-28⁺⁶ weeks' gestation, in comparison to the control arm. This was associated with changes in maternal body composition in the intervention arm, including a reduction of total GWG and sum of skinfolds (SSFT) throughout pregnancy (Poston *et al.*, 2015). However, it remains to be determined, whether this degree of change observed in the mother has an influence in determining adiposity in offspring in early infancy which has been explored in this thesis.

1.7 Hypothesis, specific objectives and aims

1.7.1 Hypothesis

This thesis assesses the hypothesis that the *in-utero* environment influences neonatal and 6 month old infant's body composition in offspring born to obese women, which has the potential to be modified by an intensive behavioural lifestyle intervention.

1.7.2 Specific aims and objectives of this thesis

For this thesis data from the UPBEAT; an RCT assessing the influence of a dietary and physical activity intervention in obese pregnancies was used to address the following aims and hypothesis:

Aim 1: To determine the relationship between early maternal risk factors and neonatal adiposity in obese women.

Objectives;

- a. To assess the association between maternal demographic, clinical and biochemical characteristics at 15-18⁺⁶ weeks' gestation and neonatal adiposity.
- b. To assess the association between a comprehensive maternal fasting metabolic profile including candidate biomarkers and metabolome in the late 2nd trimester and neonatal adiposity.
- c. To assess whether maternal metabolic profile assessed in the 2nd late trimester acted as intermediates in any observed associations between early pregnancy maternal risk factors and neonatal adiposity.

Aim 2: To investigate the cord blood metabolic profile as a measure of the fetal metabolic response to the *in-utero* environment in offspring born to obese women.

Objectives;

- a. To assess the effect of a behavioural lifestyle intervention (UPBEAT) on the cord blood metabolic profile.
- b. To assess the role of *in-utero* exposure to early pregnancy BMI, GDM and GWG on the cord blood metabolic profile.
- c. To explore the relationship between the cord blood metabolic profile in relation to neonatal and subsequent infant body composition, growth and growth velocities at 6 months of age.

Aim 3: To determine the effect of a behavioural lifestyle intervention in obese pregnancies on infant outcomes at 6 months of age.

Objectives;

- a. To assess the effect of a behavioural intervention on measures of infant adiposity at 6 months of age.
- b. To examine whether the antenatal intervention had sustained effects on maternal diet, physical activity as well as known postnatal determinants of childhood adiposity.

Aim 4: To determine the influence of early life feeding patterns on infant body composition.

Objectives;

- a. To assess whether mode of early feeding influenced infant body composition at 6 months.
- b. To determine whether early introduction of solids influenced infant anthropometry at 6 months of age.
- c. To assess whether appetite and satiety responsiveness differed by early life feeding method at 6 months of age.
- d. To investigate the relationship between measures of infant appetite and satiety and infant anthropometry at 6 months of age.

Chapter 2 Methods

2.1 Study population

2.1.1 *UK Pregnancies Better Eating and Activity Trial*

Analyses presented in this thesis were performed using data from the UPBEAT randomised controlled trial (ISRCTN89971375; Chief Investigator Professor Lucilla Poston). This multicentre, RCT was designed to evaluate a complex behavioural intervention in obese pregnant women, focusing on provision of physical activity and dietary advice, to reduce the incidence of maternal GDM and delivery of LGA infants. The study was conducted in accordance with the Medical Research Framework for the development and evaluation of RCTs for complex interventions to improve health (Craig *et al.*, 2008). The UPBEAT study comprised of three stages: Phase I; trial development and literature review, Phase II, a pilot RCT which commenced in May 2011, and the full Phase III RCT which completed recruitment (n=1555) in August 2014 (Poston *et al.*, 2015). NHS Research Ethics Committee approval was obtained at all centres (UK Integrated Research Application System; reference 09/H0802/5). All aspects of the trial, including data collection, monitoring and analysis were overseen by members of the UPBEAT consortium and an external Trial Steering Committee.

2.1.2 *UPBEAT hypothesis*

The primary hypothesis of the UPBEAT RCT was to assess a lifestyle, antenatal intervention aimed at promoting a low glycaemic diet and increasing physical activity, to reduce the incidence of maternal GDM (primary maternal outcome) and delivery of a LGA neonate (primary neonatal outcome). A secondary pre-defined hypothesis of the study, was that the intervention will reduce adiposity in the infant (Briley *et al.*, 2014).

2.1.3 *UPBEAT study endpoints*

The primary maternal aim of the study was to determine whether the intervention, delivered in the early 2nd trimester over an eight week period, was associated with a reduction in the incidence of GDM at 27⁺⁰–28⁺⁶ weeks' gestation diagnosed by an OGTT using the IADPSGs' diagnostic criteria; fasting glucose ≥ 5.1 mmol/L and/or 1 hour glucose ≥ 10.0 mmol/L; 2 hour glucose ≥ 8.5 mmol/L. For the neonate, the primary objective was to determine whether the intervention led to fewer deliveries of LGA infants defined as birthweight $\geq 90^{\text{th}}$ using a customised centile adjusted for maternal height, weight, ethnicity, parity, gestation at delivery, birthweight and neonatal sex (Gardosi and Francis, 2014).

2.1.4 *UPBEAT study population*

Obese women were recruited from 8 tertiary maternity units, located within inner city populations across the UK at 15-18⁺⁶ weeks' gestation. These included, London (St Thomas's Hospital, St Georges' Hospital and King's College Hospital), Newcastle University/ Newcastle NHS Foundation Trust, Glasgow University and Greater Clyde Health Board, Central Manchester Hospital Foundation Trust, City Hospital Sunderland and Bradford Royal Infirmary. Obese women were identified at the first hospital antenatal appointment or from midwife and General Practitioner referral letters.

Women were eligible for the study if they had a BMI $\geq 30\text{kg/m}^2$ and a singleton pregnancy at 15-18⁺⁶ weeks' gestation. Women were excluded if unable to provide informed consent, suffered from gestational hypertension requiring treatment, pre-existing renal disease, systematic lupus erythematosus, antiphospholipid syndrome, sickle cell disease, thalassemia, coeliac disease, thyroid disease, current psychosis, multiple pregnancy, pre-existing type 1 or 2 diabetes and/ or currently prescribed metformin.

2.1.4.1 Sample size

1546 women (including an allowance for 20% drop-out) (773 per arm) were required for recruitment to provide 80% power to detect a 25% reduction in the incidence of GDM. For the delivery of LGA neonates; to achieve a 30% relative risk reduction from a calculated estimate of 17.5% to 12.0% (the incidence of LGA within the pilot study was 15.8%) within the intervention arm; 1546 women would provide 80% power.

2.1.5 *UPBEAT randomisation procedures*

Women were randomised using a secured, online data repository (MedSciNet™), where data was stored. The randomisation procedure was minimised according to the women's ethnicity, parity (0 vs. ≥ 1), age and BMI (BMI class 30-34.9 kg/m² vs. 35-39.9 kg/m² vs. >40 kg/m²). Women were either randomised to the UPBEAT intervention with standard antenatal care according to local clinical guidelines or standard antenatal care alone according to local guidelines.

2.1.5.1 Components of the intervention and delivery

The UPBEAT intervention was devised on the basis of psychological models of health behaviour including control theory and social cognitive theory (Poston *et al.*, 2013). These models incorporate self-regulation techniques suggesting that behaviour change should be encouraged by continual feedback regarding performance together with the development of pre-specified goals. The lifestyle intervention was delivered via weekly sessions over an eight week period at 15-18⁺⁶ and 27-28⁺⁶ weeks' gestation by a health trainer, at which "SMART" (Specific, Measurable, Achievable, Relevant and Time Specific) goals were set. Through weekly contact sessions with a health trainers, barriers to behaviour change were addressed through provision of social support facilitated by group sessions. For women,

that were unable to attend the weekly sessions, the session content was delivered via phone or email.

Obese pregnant women were encouraged to increase consumption of foods with a lower dietary glycaemic index through exchanging foods rather than limit energy intake per se. Furthermore, advice was provided to replace foods with high saturated fat content with unsaturated fats and replacement of sugar sweetened beverages with low glycaemic index alternatives. As well as dietary advice, women were encouraged to increase daily physical activity by setting incremental step counts (aided by a pedometer) and recommended to maintain the same level of physical activity following the intervention period.

2.1.6 UPBEAT study assessment time points

Women were assessed at three time points during pregnancy; 15-18⁺⁶, 27-28⁺⁶, 34-36 weeks' gestation and 6 months postpartum. Offspring were followed up within 72 hours of birth and at 6 months postpartum. The study protocol is summarised in Figure 2.1.

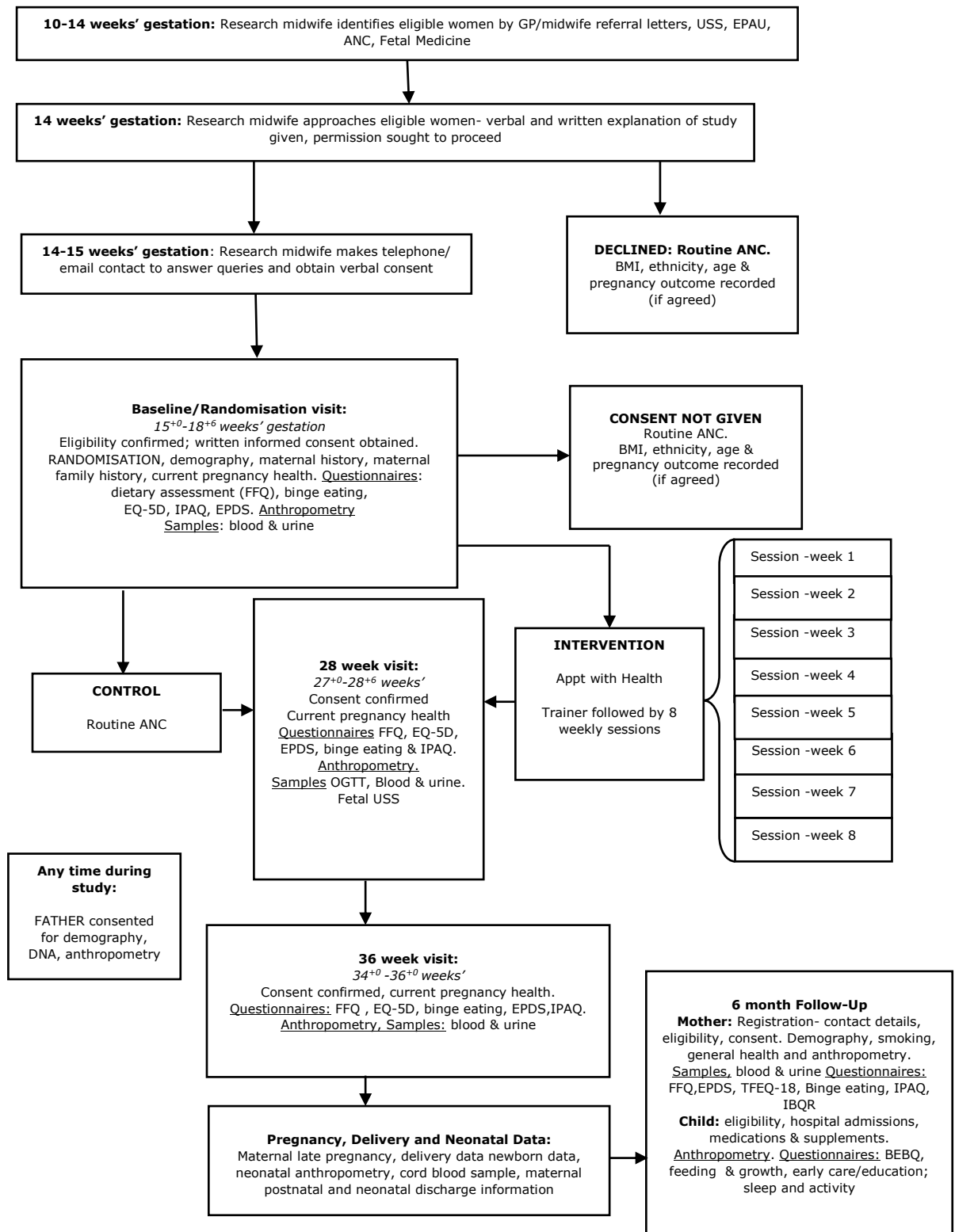


Figure 2.1: Summary of the UK Pregnancies Better Eating and Activity Trial protocol.

Abbreviations: ANC; Antenatal clinic, BEBQ; baby eating behaviour questionnaire, BMI; Body mass index, DNA; Deoxyribonucleic acid, EPAU; Early pregnancy assessment unit, EPDS; Edinburgh postnatal depression score questionnaire. EQ-5D; EuroQuo-5 European Quality of life questionnaire-5; FFQ; Food frequency questionnaire, GP; General Practitioner, IBQ-R; Infant behaviour questionnaire revised; IPAQ; International physical activity questionnaire; LGA; Large for gestational age OGTT; Oral glucose tolerance test, SGA; Small for gestational age, TFEQ-18; Three-factor eating questionnaire-R18, USS; Ultrasound scanning.

At trial entry, detailed maternal demography, familial environment characteristics, medical and family history were collected. A short-validated FFQ was completed to evaluate dietary glycaemic load, glycaemic index, saturated fat, total sugar and other dietary variables at each UPBEAT study assessment visit. Anthropometric measurements including SFTs and circumferences together with the collection of blood and urine samples. Behavioural and psychological measures were assessed by questionnaire at each study visit. Women, irrespective of randomisation allocation were invited to attend for an OGTT at 27-28⁺⁶ weeks' gestation, having fasted for a minimum of 10 hours. Women were provided with a 75gram glucose load and diagnosis of GDM was made in accordance to the IADPSG's diagnostic criteria (Metzger *et al.*, 2008).

Late pregnancy data was collected from hospital medical records including 3rd trimester maternal health, mode of delivery and number of antenatal inpatient nights. Neonatal outcomes, including collection of cord blood samples and detailed anthropometric measurements were collected within 72 hours of birth where possible.

Mothers and infants were followed up at 6 months postpartum. Mothers' pregnant at the time of the postpartum visit were excluded from the study but not their children. Infants were excluded from the follow-up visit if suffering from a major medical condition at 6 months of age. Detailed demography, clinical, anthropometric (maternal and infant), lifestyle and biochemical variables were recollected at this visit. Known determinants of childhood obesity were also collected including a detailed feeding and growth questionnaire, a validated questionnaire addressing appetite (Baby Eating Behaviour Questionnaire), a revised infant behaviour questionnaire (IBQ-R), childcare and sleep patterns. Data collected including relevant questionnaires for both mothers and their offspring are summarised in Table 2.1.

Data collected	Questionnaire where appropriate	15-18 ⁺⁶ weeks' gestation	27-28 ⁺⁶ weeks' gestation	34-36 weeks' gestation	Birth	6 months postpartum
Maternal						
Demography (Including socioeconomic status)		X				X
Diet	FFQ & 24 hour recall	X	X	X		X
Physical activity	IPAQ	X	X	X		X
Clinical history	Hospital admissions	X	X	X	X	X
Clinical assessment		X	X	X	X	X
Anthropometry		X	X	X		X
Biochemical analyses		X	X			
Metabolomics analysis		X	X	X		X
Neonate						
Clinical history	Hospital admissions				X	
Clinical assessment					X	
Anthropometry					X	
Metabolomics (cord)					X	
Infant						
Clinical history	Hospital admissions					X
	Medicines & supplements					X
Anthropometry						X
Diet	Feeding and growth					X
	BEBQ					X
Lifestyle	Childcare					X
	Sleep					X

Table 2.1: Data collected throughout the UK Pregnancies Better Eating and Activity Trial.

Including questionnaires and biochemical samples collected for mother and offspring throughout the UPBEAT study to 6 months postpartum. Abbreviations; BEBQ- Baby Eating Behaviour Questionnaire; FFQ- Food Frequency Questionnaire; IPAQ- International Physical Activity Questionnaire.

I joined the UPBEAT group following completion of the trial and 6 month follow up. My involvement included data monitoring of the study database followed by extensive data management including development of the data dictionary, devising a statistical analysis plan, a major role in the main trial analysis and preparation of the trial outcome manuscript for publication.

2.1.7 UPBEAT data management

2.1.7.1 UPBEAT data monitoring

Weekly reports were generated to aid the midwives in following up missing data and checking inconsistencies within the data (Figure 2.2). Throughout the data monitoring, randomisation allocation was masked by generation of a random number sequence. If inconsistencies in the data were identified, the data was discussed with the appropriate midwife team in detail and where possible the data was rechecked with clinical notes at the individual centres.

Table 3: Maternal primary endpoint by centre									
	STH	CAN	NC	GLA	MAN	BRI	SUN	SGH	TOTAL
OGTT performed	320	211	207	224	121	45	78	94	1300
OGTT not performed	2	0	0	2	1	1	0	0	6
Missing	62	67	35	39	17	7	6	17	250
Total	384	278	242	265	139	53	84	111	1556

Table 4: Neonatal primary end point by centre									
	STH	CAN	NC	GLA	MAN	BRI	SUN	SGH	TOTAL
Recorded	364	262	223	260	135	51	80	85	1460
Missing	11	11	18	2	3	1	3	24	73
LTFU	4	2	0	2	1	0	0	1	10
MISC <23 ⁴⁶	5	3	1	1	0	1	1	1	13
Total	384	278	242	265	139	53	84	111	1556

Figure 2.2: Data monitoring; example of missing data report by trial centre.

These reports were generated on a weekly basis to assessing missing data stratified by centres across the UK.

2.1.7.2 Data dictionary at six months

The UPBEAT RCT and 6 month follow-up collected >6000 variables. To fully utilise the database and available data, a data dictionary was created (an extract of the data dictionary is shown in Figure 2.3), by merging the individual datasets corresponding to each study visit. Furthermore, new names were derived for the variables by the addition of the prefix to identify the time point and type of participant (for example maternal body weight at 6 months postpartum; “*bodyc_weight*” changed to “*f9b_bodyc_weight*”) to ease the import of data in statistical software packages and analyses. The dictionary incorporated all variables collected, including dietary, physical activity and biochemical data. Details were also provided on type of data and the % missing, to determine missingness of variables.

1	Variable name as exported	Variable name	Variable explanation	Variable options
2	childid	childid	Participant ID (linked to maternal ID in main UPBEAT outco	continuous
3	centre	centre	Recruiting centre	1- St Thomas' 2-CAN 3-Newcastle 4-Glasgow 5-Manchester 6-Bradford 7-Sunderland 8-St Georges'
4	status	status	Study status	1- Continuing with follow up study 2- Declined to particpate
5	contact__letter	f9_contact__letter	Maternal participant contact made by letter	1- Yes; 2-No
6	contact__letter_date	f9_contact__letter_date	Date of contact letter	Date
7	contact__email	f9_contact__email	Maternal participant contact made by email	1- Yes; 2-No
8	contact__email_date	f9_contact__email_date	Date of contact email	Date
9	contact__tel	f9_contact__tel	Maternal participant contact made by telephone	1- Yes; 2-No
10	contact__tel_date	f9_contact__tel_date	Date of contact telephone	Date
11	contact__text	f9_contact__text	Maternal participant contact made by text	1- Yes; 2-No
12	contact__text_date	f9_contact__text_date	Date of contact text	Date
13	contact__mother_responded	f9_contact__mother_responded	Mother resonded to contact made	1- Yes; 2-No
14	contact__mother_respond_date	f9_contact__mother_respond_date	Date mother responded to contact	1- Yes; 2-No
15	contact__child_age_years_	f9_contact__child_age_years_	Age of child when mother responded in years	Continuous
16	contact__child_age_months_	f9_contact__child_age_months_	Age of child when mother responded in months	Continuous
17	contact__alive_when_contacted	f9_contact__alive_when_contacted	Child alive when mother contacted	1- Yes; 2-No
18	contact__major_health_problems	f9_contact__major_health_problems	Child- major health problems at 6 months	1- Yes; 2-No
19	contact__major_health_problems_	f9_contact__major_health_problems_	Freetext of major health problems	Free text
20	contact__eligible	f9_contact__eligible	Mother and child eligible to participate based on age	1- Yes; 2-No
21	contact__agreed_to_participate	f9_contact__agreed_to_participate	Mother/ child agreed to participate within UPBEAT follow u	1- Yes; 2-No
22	contact__reason_if_did_not_agre	f9_contact__reason_if_did_not_agre	Reasons of declining to participtate	Free text
23	contact__comments	f9_contact__comments	Further comments regarding contact	Free text
24	reg__current_maternal_initials	f9_reg__current_maternal_initials	Maternal initials at registration of follow up study	Free text
25	reg__child_initials	f9_reg__child_initials	Child initials at registration of follow up study	Free text

Figure 2.3: An extract of the UK Pregnancies Better Eating and Activity Trial data dictionary at 6 month follow-up visit.

An extract of the data dictionary created for the UPBEAT 6 month data based on type of participant, time point of data collection and data collected.

2.1.8 UPBEAT study results

The UPBEAT study did not meet its primary endpoints of reducing maternal GDM or delivery of a LGA infant (Table 2.2). The intervention was however, associated with significant changes in maternal antenatal diet, physical activity and measures of body composition. These include significant reductions in total energy intake (Mj/day) (-0.70; 95% CI -0.96 to -0.45; $p < 0.0001$), saturated fat (% energy) (-0.85; 95% CI -1.2 to -0.51; $p < 0.0001$) and glycaemic load per day (-21; 95% CI -26 to 16; $p < 0.0001$) and increased physical activity as assessed by the MET (min/week) (295; 95% CI 105 to 485; $p = 0.0015$) from 15-18⁺⁶ to 26-28⁺⁶ weeks' gestation, in comparison to the control arm. There were also associated changes in maternal body composition in the intervention arm, including a reduction of total GWG (kg) (mean difference -0.55; 95% CI 1.08 to -0.02; $p = 0.041$) and SSFT throughout pregnancy (mean difference -3.2; 95% CI -5.6 to -0.8; $p = 0.0081$) (Poston *et al.*, 2015).

	Standard care	Intervention	Effect of Intervention	p-value
Gestational diabetes *	172/651 (26%)	160/629 (25%)	<i>Risk Ratio</i>	0.68
			0.96 (0.79 to 1.16)	
			<i>Risk difference</i>	
			-1.2% (-5.8% to 3.8%)	
Large for gestational age $\geq 90^{\text{th}}$ centile**	61/751 (8%)	71/761 (9%)	<i>Risk ratio</i>	0.40
			1.15 (0.83 to 1.59)	
			<i>Risk difference</i>	
			1.2% (-1.6% to 4.1%)	

Table 2.2: UK Pregnancies Better Eating and Activity Trial primary outcomes by randomisation to standard care and intervention arms.

*Primary maternal outcome: Gestational diabetes diagnosed at 27+0 to 28+6 weeks' gestation by oral glucose tolerance test by IADPSG criteria¹⁴. Included were 8 (4.7%) of women with a diagnosis of gestational diabetes in the control group and 10 (6.3%) in the intervention group whose glucose tolerance test was undertaken within 6 days, (mean 3.0) of 27+0 to 28+6 weeks. A sensitivity analysis using only tests within the pre-defined window showed similar results (intervention 26.5% vs. control 25.5%; risk ratio 0.96, 95% confidence intervals 0.79 to 1.16; $p = 0.67$). **Neonatal outcome defined using customised centiles (Gardosi and Francis, 2014). Source: Poston L *et al.* Effect of a Behavioural Intervention in Obese Pregnant Women (the UPBEAT study): A Multicentre Randomised Controlled Trial. *Lancet Diabetes & Endocrinology* 2015;3:767-777 (Poston *et al.*, 2015).

2.2 Study design

For this thesis, two predominant study designs were utilized; RCT and cohort design. In this thesis, the study design used was based on the hypothesis of interest and the aims and objectives of each analysis.

2.2.1 Randomised controlled trials

Within this study design, participants are randomly allocated to a treatment group and outcomes of interest are followed up. RCTs are regarded as the ‘gold standard’ to establish causal association (Richmond *et al.*, 2014). Within a well-designed study, randomisation ensures that any imbalance between the two groups at baseline is purely due to chance, thereby limiting potential confounding.

2.2.2 Cohort studies

Cohort studies are observational studies which seek to investigate causes of disease within an unselected population. As cohort studies are observational studies, there may be influenced by confounding and bias.

2.3 Primary outcome of this thesis

The primary outcome of interest in this thesis was neonatal and infant adiposity as assessed by skinfold thicknesses in neonates and infants at 6 months of age.

SFTs were collected within 72 hours of birth in neonates, and at 6 months of age in the infants. All measurements were collected in triplicate. Details of methods of measurement collection are summarised in Table 2.3.

Offspring anthropometric measurement		Methodology
Neonates		
Skinfold thickness	Triceps (mm)	Using the Harpenden skinfold callipers, measured at the posterior aspect of the arm over the triceps muscle, at a midway point between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna.
	Subscapular (mm)	Using the Harpenden skinfold callipers, measured at the inferior lateral border of the scapula.
Birthweight (grams)		Defined as the first weight recorded after delivery in grams.
Length (cm)		Measured using the neonatometer
Midarm circumference (cm)		Circumference measured midway between the elbow and shoulder bone.
Abdominal circumference (cm)		Circumference measured at the point of the umbilicus.
Occipitofrontal circumference (cm)		Circumference measured at the maximal Occipitofrontal diameter with the neonate supine.
Infant at 6 months		
Skinfold thicknesses	Triceps (mm)	Using Holtain callipers, measured at the posterior surface of the triceps muscle.
	Subscapular (mm)	Using Holtain callipers, measured at the lower most tip of the scapula.
Length (cm)		Measured with the infant in the supine position with his/her back flat on the infantometer.
Weight (grams)		Measured using SECA scales ideally weight naked.
Abdominal circumference (cm)		Circumference measured at the level of the umbilicus
Upper midarm circumference (cm)		Circumference measured at the midpoint of the upper left arm.

Table 2.3: Offspring anthropometric measurements and associated methodology for assessing neonates and infants at 6 months of age in the UPBEAT study.

All staff underwent rigorous training for the measurement of neonatal SFTs. Intra-observer and intra-subject variations were continually assessed at each participating centre by collaborators at the University of Southampton. Assessment was undertaken by fitting analysis of variance models looking at the effect of staff and order. Results were visualised graphically to determine if the differences were clinically important. An example of intra-observer variations by two research midwives at a particular centre are shown in Figure 2.4 for neonatal subscapular SFTs. There was no significant difference between research staff at each of the centres and the order of measurements undertaken.

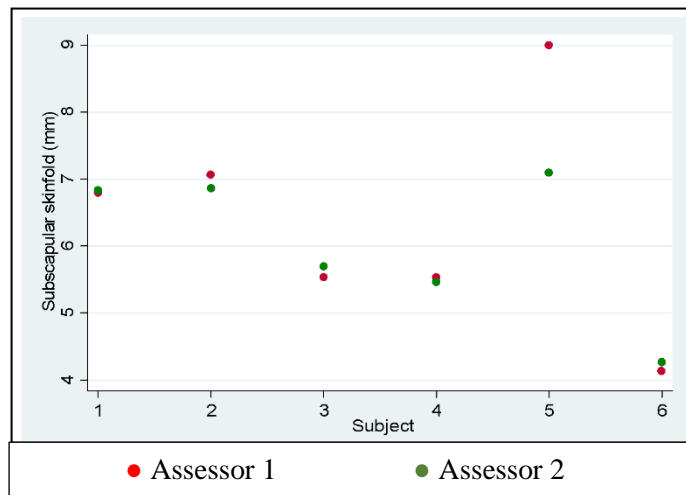


Figure 2.4: Intra-observer variation in assessment for neonatal subscapular skinfold thicknesses.

2.3.1 *Derived measures of offspring adiposity*

Neonatal SSFTs were calculated by the addition of triceps and subscapular SFTs. For the infant at 6 months of age, SSFTs were calculated as the addition of triceps and subscapular SFTs and individual SFTs (subscapular and triceps SFTs) z-scores were determined using the WHO reference population (WHO, 2006). This reference population adjusts for offspring sex, age and is applicable irrespective of ethnicity and mode of early infant feeding.

2.4 **Secondary outcomes**

2.4.1 *Neonatal secondary outcomes*

Secondary neonatal outcomes included mid-upper arm circumference (methodology for collection summarised in Table 2.3) and birthweight z-score calculated using external statistics derived from UK population data taking into account sex and gestation at delivery using the following formula (Cole *et al.*, 2011);

$$z = \frac{(x-m)^{l-1}}{(l \times s)},$$

where z -standard deviation score; x -observed weight; and l, m, s are parameters dependent on gestation at delivery & offspring sex (Cole *et al.*, 2011).

2.4.2 Infant secondary outcomes at 6 months of age

Infant secondary outcomes are summarised in Table 2.4.

Variable generated	Variable type	Definition
Weight for length z-scores	Continuous	Generated using growth standards from the WHO Anthro (de Onis and Blössner, 2003)*
Infant body mass index z-scores	Continuous	Generated using growth standards from the WHO Anthro (de Onis and Blössner, 2003)*
	Binary	BMI z-score $\geq 85^{\text{th}}$ centile- infant BMI subcategorised as per WHO thresholds (de Onis and Blössner, 2003).
	Binary	BMI z-score $\geq 95^{\text{th}}$ centile- infant BMI subcategorised as per WHO thresholds.
Length for age z-score	Continuous	Generated using growth standards from the WHO Anthro (de Onis and Blössner, 2003)*
Occipitofrontal for age z-score	Continuous	Generated using growth standards from the WHO Anthro (de Onis and Blössner, 2003)*
Midarm circumference for age z-score	Continuous	Generated using growth standards from the WHO Anthro (de Onis and Blössner, 2003)*
Catch up growth	Binary	An increase in of $>0.67^{\text{I}}$ standard deviations of weight from birth to 6 months of age was defined as clinically significant catch up growth at 6 months (de Onis and Blössner, 2003).
Catch down growth	Binary	A decrease of $>0.67^{\text{I}}$ standard deviations of weight from birth to 6 months of age was defined as clinically significant catch down growth a 6 months (de Onis and Blössner, 2003).

Table 2.4: Derived measures of infant body composition and methodology for calculation of measures used within the presented analyses.

**The WHO Anthro z-scores are calculated considering the infant's age (days) and sex. Biological implausible values are highlighted for each anthropometric z-score and are defined as ± 5 SDS with the exception for infant length (± 6 SDS). Biological implausible values were dropped from the analysis.*

Estimated total body (%) for infants at 6 months was also calculated from 2 skinfold measurements (triceps and subscapular SFT). Total body fat estimation was calculated using cross-validated skinfold equations (Slaughter *et al.*, 1988) using the addition of offspring triceps and subscapular SFTs;

$$\text{Male infants body fat (\%)} = 1.21(\Sigma 2 \text{ SFT}) - 0.008(\Sigma 2 \text{ SFT}) - 1.7$$

$$\text{Female infants body fat (\%)} = 1.33 (\Sigma 2 \text{ SFT}) - 0.013 (\Sigma 2 \text{ SFT}) - 2.5$$

2.5 Exposures

2.5.1 Maternal in-utero exposures

2.5.1.1 Demographic variables

At trial registration, maternal age and pre-pregnancy BMI were collected at 15-18 weeks' gestation. Inconsistencies were checked with recorded date of birth and objectively assessed maternal weight and height at trial entry. Clinical factors for example maternal parity, educational attainment (≤ 11 years vs. > 11 years of full time education) or ethnicity (White vs. Black vs. Asian vs. Other) were generated as binary and categorical variables, where appropriate. Maternal smoking, was generated as both binary (current smoker vs. non-smoker) and categorical (current smoker in early pregnancy vs. gave up smoking in early pregnancy vs. ex-smoker vs. non-smoker).

Socio-economic deprivation was measured using the Lower Super Output Area which was collected from participants and corresponded to the census region of the participant's residence. These were subsequently mapped to the corresponding index of multiple deprivation quintiles and subscales. As the index of multiple deprivation scores are not directly comparable to the four constituents regions of the UK, a methodology proposed by Payne et al was used to combine the four regions; allowing generation of an adjusted index of multiple deprivation score using Scottish Index of Multiple Deprivation as a baseline (Payne and Abel, 2012). Through publicly available data; an adjusted index of multiple deprivation score was created using employment and income domains of the individual country together with coefficients and residual values, generated by regression analyses of the overall index of multiple deprivation score and employment and income data.

2.5.1.2 Family history of medical disorders.

Family history of cardiometabolic health and pregnancy history were collected for all first-degree relatives. The data was collapsed to generate individual variables for any maternal, paternal or sibling history of cardiometabolic disease and pregnancy outcomes including previous GDM or pre-eclampsia where appropriate.

2.5.1.3 Maternal anthropometric measurements

Methods for anthropometric data collection for mothers are summarised in Table 2.5. SFTs and circumferences were collected in triplicates by trained midwives throughout pregnancy.

Maternal Anthropometric measurement		Methodology
Waist circumference		Circumference measured at the half way point between the iliac crest and inferior margin of the lowest rib.
Hip circumference		Circumference from the maximal diameter of the buttocks.
Thigh circumference		Circumference measured from the observed largest part of the thigh.
Mid arm circumference		Circumference measured from the midway point between the elbow and apex of the shoulder.
Skinfold thicknesses	Triceps	Using Harpenden skinfold callipers, measured at the posterior aspect of the arm over the triceps muscle, at a midway point between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna.
	Biceps	Using the Harpenden skinfold callipers, measured at the anterior aspect of the arm over the biceps muscle with the upper extremity relaxed to the side.
	Subscapular	Using the Harpenden skinfold callipers, measured at inferior lateral border of the scapula with the calliper jaws placed infero-laterally, 45° to the horizontal plane.
	Suprailiac	Using the Harpenden skinfold callipers; measured above the crest of the ilium.

Table 2.5: Maternal anthropometric measurements and associated methodology for collection in the UPBEAT study.

2.5.1.3.1 Gestational weight gain

Maternal weight was objectively recorded at three time points during pregnancy (15⁺⁰ to 18⁺⁶, 27⁺⁰ to 28⁺⁶, 34⁺⁰ to 36⁺⁰ weeks and 6 months' post-partum). Total GWG for this

cohort was defined as total weight gained from a calculated pre-pregnancy weight to late third trimester.

As pre-pregnancy weight was subjectively obtained from medical notes, an assumed fixed first trimester weight gain of 1.25kg as recommended by the IOM weight gain guidelines was used to more accurately estimate pre-pregnancy weight. 1.25kg is the midpoint of the range specified by the IOM guidelines (0.5 to 2.0kg) (Rasmussen and Yatkine, 2009). In order to estimate as accurately as possible, 1.25kg was subtracted from each objectively measured maternal weight at 15⁺⁰ to 18⁺⁶ weeks' of gestation, to generate an estimated pre-pregnancy weight (Fall and Ingelsson, 2014). Total GWG was defined as the last recorded weight measured prior to delivery minus the derived pre-pregnancy weight.

2.5.1.4 Maternal clinical factors

The methodology described below includes principles of data management and statistical analyses applied to the pre-specified maternal and infant clinical factors taken within the trial and at six months postpartum. Maternal clinical variables and their definitions, which were generated from the original data are shown in Table 2.6.

Variable generated		Variable type	Definition
Gestational diabetes	26-28 ⁺⁶ weeks' gestation	Binary	Defined as fasting glucose ≥ 5.1 mmol/L; and/or 1 hour glucose ≥ 10.0 mmol/L; and 2 hour glucose ≥ 8.5 mmol/L following a 75g glucose load and oral glucose tolerance test undertaken in late second trimester
Pre-eclampsia		Binary	Pre-eclampsia/ severe pre-eclampsia was defined as new hypertension presenting after 20 weeks associated with significant proteinuria. Severe pre-eclampsia was defined as pre-eclampsia with severe hypertension and/or with symptoms, and/or biochemical and/or haematological impairment. Both variables are collected retrospectively and therefore treated as binary outcome data.
Antepartum haemorrhage		Continuous/ Binary	Subcategorised to blood loss at delivery ≥ 1000 ml and ≥ 2000 mls.
Mode of delivery		Categorical	Categorised to vaginal vs. instrumental vaginal vs. elective C-section vs. emergency C-section.
Hospital inpatient hospital nights		Continuous	Defined as total number of days admitted into hospital for ≥ 24 hours.
		Binary	Number of inpatient nights ≥ 1 vs. <1 .

Table 2.6: Definitions of derived maternal clinical variables used throughout the UPBEAT study.

2.5.2 Maternal dietary and physical activity data

2.5.2.1 Dietary recording

Dietary intake of the UPBEAT participants was assessed using FFQs administered by research midwives at 15-18⁺⁶, 27-28⁺⁶ and 34-36⁺⁰ weeks' gestation. In addition, the same FFQ was used to assess maternal dietary intake at 6 months postpartum.

The UPBEAT FFQ is an adaptation of the questionnaire used for the European Prospective Investigation into Cancer study in the UK (Bingham *et al.*, 2001; Riboli *et al.*, 2002), consisting of 50 questions to establish average use of foods during the preceding month.

The questionnaire consisted of two parts; Part 1, food types including milk, breakfast

cereal, bread, butter and cheese; Part 2, food groups, included as individual or groups of foods. Accompanying the list was a multiple response grid in which the respondents estimated frequency of consumption of foods eaten over the last month, ranging from never or less than once a month to 6 or more times per day. An automated programme was developed by the trial database team to transform data from the food frequency questionnaires into nutrient intakes based on the Compositional Analyses developed for European Prospective Investigation into Cancer Study nutritional data analysis (Riboli *et al.*, 2002). Single food and beverage items listed in Part 1 of the questionnaire were assigned food codes and combination of foods were selected from food composition tables representing an average nutrient composition for each group (Robertson, 2003). Food codes were entered into WISP 3.0 (Tinuviel Software) dietary analysis software which produced nutritional composition and glycaemic load per 100g for each food code. Each glycaemic load value was manually checked by the trial dietician and updated glycaemic load values were assigned using published glycaemic index tables (Foster-Powell *et al.*, 2002; Aston *et al.*, 2010) .

Using principal component analysis, four dietary patterns were developed defined as ‘Fruit and vegetable’, ‘Processed’, ‘African/Caribbean’ and ‘Snacks’ (Figure 2.5) (Flynn *et al.*, 2016). Women with unusually high and low energy intakes (>4780 Kcal and < 1780 Kcal) at 15-18 weeks’ gestation were omitted in accordance with previously reported analyses (Bingham *et al.*, 2001; Flynn *et al.*, 2016).

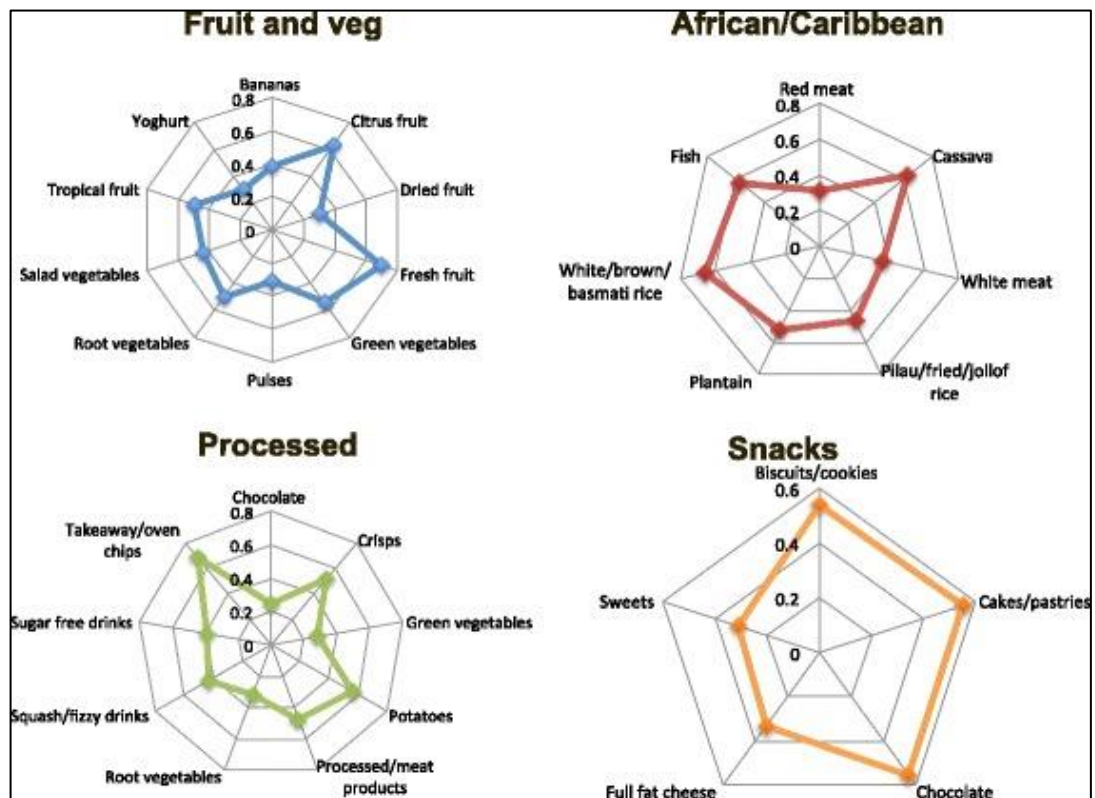


Figure 2.5: Maternal dietary patterns at 15-18⁺⁶ weeks' gestation from the UK Pregnancies Better Eating and Activity study.

Dietary patterns derived by principal component analysis and factor loadings ≥ 0.25 were considered significant. Source: Flynn AF, Goff L, Seed PT, Patel N et al. Dietary Patterns in Obese Pregnant Women; Influence of a Behavioural Intervention of Diet and Physical Activity in the UPBEAT Randomized Controlled Trial. International Journal of Behavioural Nutrition and Physical Activity 2016;13:124-128 (Flynn et al., 2016).

2.5.2.2 Physical activity

Maternal physical activity was assessed throughout pregnancy and at 6 months postpartum by the short form of the International Physical Activity Questionnaire (IPAQ) (Ekelund *et al.*, 2006). The self-administered questionnaire collected information on frequency and duration of three types of physical activity including walking, moderate and vigorous intensity physical activity over the previous 7 days.

The IPAQ was summarised in accordance with published guidance (Ekelund *et al.*, 2006).

Outliers were defined as data which were unreasonably high; for example, physical activity data ≥ 16 hours/ day and identified observations were dropped from the analysis. According

to the IPAQ guidelines, walking, moderate and vigorous activity time >4 hours/ 240 minutes were recoded to equal 4 hours/ 240 minutes; to take into account subjective bias (Ekelund *et al.*, 2006). To summarise total physical activity, MET was generated, using the following formula:

$$MET = (walking(mins\ per\ week) \times 3.3) + (moderate\ activity\ (mins\ per\ week) \times 4.0) + (vigorous\ activity\ (mins\ per\ week) \times 8.0)$$

MET scores were subcategorised using the following pre-defined criteria below (Ekelund *et al.*, 2006):

- a. *High physical activity*; vigorous activity on at least 3 days per week achieving a total physical activity of at least 1500 MET in minutes per week or a combination of walking, moderate activity or vigorous activity on 7 days per week, achieving a total physical activity of at least 3000 MET minutes per week.
- b. *Moderate physical activity*; 3 or more days per week of vigorous intensity activity of at least 20 minutes per day or 5 or more days per week of moderate intensity activity or walking for at least 30 minutes per day or achieving total physical activity of at least 600 MET in minutes per week.
- c. *Low physical activity*; participants not meeting the moderate or high physical activity criteria will subsequently be allocated to the low activity group.

2.5.3 Neonatal birth outcomes

Neonatal outcomes collected from maternal medical records and Electronic Patient Records™ including gestation at delivery, admission to neonatal intensive care and offspring sex. Gestation at delivery was subcategorised to preterm delivery (≤ 37 weeks' gestation vs. >37 weeks gestation).

2.5.4 *Candidate biochemical markers*

Maternal venous samples were collected at each study time-point and underwent biochemical and metabolomics analyses at the University of Glasgow and Brainshake Ltd® respectively. Mixed-blood cord samples were collected at the time of delivery for metabolomic (Ludwig Maximilian, University of Munich) and candidate biomarker analyses (University of Glasgow). The gestation at sample collection was calculated at each time-point using the exact date that the blood sample was taken. The following definitions were used to create the variables

1. *15-18⁺⁶ weeks' study visit.* Gestation at randomisation to the UPBEAT intervention calculated from gestational age at trial entry.
2. *27-28⁺⁶ weeks' study visit.* Gestation calculated from date at sample collection minus gestational age at trial entry.
3. *Cord metabolome.* Taken as gestation at delivery.

2.5.4.1 Maternal biochemical markers

Maternal biochemical analyses undertaken at the time of writing this thesis are summarised, together with appropriate methodology, in Table 2.7.

Maternal Biochemical variable	15-18 ⁺⁶ weeks' gestation	27-28 ⁺⁶ weeks' gestation	Methodology
	<i>Random sample</i>	<i>Fasting sample</i>	
Serum targeted metabolome	X	X	High-throughput serum nuclear magnetic resonance metabolomics platform was used simultaneously quantify lipids, 14 lipoprotein subclasses and major fractions, fatty acids, amino acids, ketone bodies and gluconeogenesis related metabolites; all previously implicated with insulin resistance.
Total cholesterol	X	X	Enzymatic, colorimetric using Roche, Cobas c311 (CV low 1.8%, high 1.1%)
LDL cholesterol	X	X	Homogenous enzymatic colorimetric using Roche Cobas c311 (CV low 2.0%)
HDL cholesterol	X	X	Homogenous Enzymatic, colorimetric using Roche Cobas c311 (CV low 2.0%)
Glucose	X	X	Enzymatic, hexokinase using Roche Cobas c311
Fructosamine	X	X	Colorimetric, nitroblue tetrazolium using Roche Cobas c311 (CV low 3.4%)
HbA1c	X		Turbidimetric inhibition immunoassay using Roche Cobas c311 (CV low 1.4%, high 1.3%)
Insulin	X	X	Electrochemiluminescence immunoassay using Roche Cobas c411 (CV low 7.8%, high 5.4%)
C-peptide	X	X	Electrochemiluminescence immunoassay using Roche Cobas c311 (CV low 6.2%, high 5.1%)
hs-CRP	X	X	Particle enhanced immunoturbidimetric using Roche Cobas c411 (CV low 7.15)
gGT	X	X	Enzymatic, colorimetric using Roche Cobas c311 (CV low 3.9, high 3.9%)
ALT	X	X	Enzymatic, spectrophotometric using Roche Cobas c311 (CV low 3.3%, high 3.1%)
AST	X	X	Enzymatic, spectrophotometric using Roche Cobas c311 (CV low 2.1%, high 1.8%)
Triglycerides	X	X	Enzymatic, colorimetric using Roche Cobas c311 (CV low 2.2, high 1.8%)
Leptin	X	X	Enzyme-linked immunosorbent assay using R&D systems (CV intra 2.0%, inter 9.3%)
Adiponectin	X	X	Enzyme-linked immunosorbent assay using R&D systems (CV intra 5.4%, inter 12.0%)
IL-6	X	X	Enzyme-linked immunosorbent assay using R&D systems (CV intra 9.8%, inter 12.8%)

Table 2.7: Maternal biochemical analyses undertaken including specific candidate biomarkers.

Abbreviation; CV- Coefficient of variation.

2.5.4.2 Cord blood biochemical markers

Candidate cord biochemical analyses were chosen based on previous published research in relation to offspring adiposity and subsequent growth. Cord insulin, C-peptide and glucose were measured as these have been shown to be reflective of maternal glycaemic status and offspring adiposity (Metzger *et al.*, 2009; Catalano *et al.*, 2012). Plasma insulin and C-peptide, were measured by electrochemiluminescence on a clinically validated automated platform (e411, Roche Diagnostics, Burgess Hill, UK). The adipokines leptin and adiponectin were assessed as these have been shown to correlate highly with neonatal fat mass at birth and in early infancy (Clapp and Kiess, 1998; Fairley *et al.*, 2015). Inflammatory markers IL-6 and Tumour Necrosis Factor (TNF- α) were measured, as both have been implicated as endocrine and paracrine signals to induce insulin resistance and shown to correlate with infant weight gain in the first year of life (Hotamisligil *et al.*, 1993; de Toledo Baldi *et al.*, 2016). Cord adiponectin, IL-6, TNF- α , adiponectin and leptin were measured by enzyme-linked immunosorbent assay (R&D Systems, Abingdon, U.K.). Insulin growth factors (IGF) I & II were also assessed as critical determinants of fetal and infant growth; cord blood IGF-I and IGF-II, correlated positively with birth weight and with measures of neonatal adiposity respectively (Lawlor *et al.*, 2014), and have been shown to be associated with the cord lipid profile in offspring born to type 1 diabetic mothers (Nelson *et al.*, 2007). Furthermore, three studies have reported reduced methylation status of IGF-II in obese mothers associated with increased IGF-II concentration in cord blood. IGF I and II were determined using a two-site immunoenzymometric assay (IDS, Fountain Hills, USA).

2.5.5 *Metabolome*

Metabolomics is the quantitative analysis of low molecular weight molecules within a biological tissue or fluid. Metabolites are end products of enzymatic processes and therefore are the accurate proxies of the physiological process occurring within an

organism. Metabolomic analysis thus enables the examination of metabolic signatures, providing detailed insight into physiological or pathophysiological processes. At present, there are two principal methods of metabolomics analysis; targeted or untargeted. Targeted metabolomics are typically a-priori driven, where the analysis plays focus to a finite number of molecules. In comparison, untargeted metabolomics is an unbiased approach, in which multiple metabolites are measured for the discovery of novel biomarkers in relation to a disease state or physiological process. Table 2.8 summarises the differences between the two approaches.

	Untargeted metabolomics	Targeted metabolomics
Summary	Detection of many metabolites to provide insight into multiple metabolite pathways. Provides relative metabolite concentrations.	Detection of a small number of metabolites associated with an a-priori hypothesis. Able to provide absolute metabolite concentrations.
Platform	Mass spectrometry	Mass spectrometry or nuclear magnetic resonance spectroscopy
Uses	Discovery of novel biomarkers. Provides information of numerous biological pathways.	Hypothesis testing with the ability to provide detailed insight into the related metabolic pathways.
Advantages	Discovery of novel biomarkers/ metabolites.	Shorter analysis time with high specificity and accuracy of derived metabolite concentrations.
Disadvantages	Findings may require replication using targeted approaches. Possibly low specificity and accuracy.	Potentially miss important metabolites or biological pathways.

Table 2.8: Comparison of characteristics associated with untargeted and targeted metabolome.

There are two main analytical approaches associated with metabolomics analysis; mass spectrometry (MS) and nuclear magnetic resonance (NMR). NMR spectroscopy is a highly reproducible technique requiring minimal sample preparation, which allows quantification of the concentration of the metabolites and detailed structural information. However, the main disadvantage of NMR is that smaller molecules (for example those with smaller energy transitions including amino acids), potentially may be concealed by larger molecules (for example metabolites with higher energy transitions including phospholipids and triglycerides). MS is typically coupled with an ion separation technique, for example

gas-liquid chromatography or liquid chromatography. In comparison to NMR, MS has higher sensitivity but is limited by capturing multiple structures with varying dimensions of the same metabolite, therefore resulting in over-representation of the same molecule.

For this thesis, maternal serum samples were analysed using NMR, whereas cord samples were analysed using MS. Reasons for methodology selection are discussed below.

2.5.5.1 Maternal metabolomics samples

Maternal antenatal samples were analysed using NMR profiling in collaboration with Professor Ala Korpela (Brainshake Ltd®) using a patented platform. Directly measured metabolites include lipoprotein subclasses, low-molecular-weight metabolites (amino acids, ketone bodies and creatinine) and detailed molecular information on serum lipid extracts including free and esterified cholesterol, sphingomyelin, degree of saturation and ω -3 fatty acids. This methodology was developed, internally validated and quality controlled to not exhibit machine ‘drift’ from batch to batch; thereby providing comparable results to measurement by routine assay. Previous studies have demonstrated that maternal obesity is associated with alterations in branched amino acids and degradation products related to glycolysis, however the mechanisms associated with the development of offspring adiposity born to obese mothers are unknown. For the purposes of this thesis, a targeted metabolomic analysis was undertaken to provide clear biochemical significance of metabolites associated with maternal insulin resistance and offspring adiposity.

The high throughput proton NMR metabolomics platform used, provided rapid quantification of 64 primary metabolic measures (summarised in Table 2.7) as well as over >100 derived measures. The platform quantifies metabolites using a 600MHz spectrometer. These spectra were recorded, each yielding different subclasses of metabolites;

1. *Window 1 (LIPO window)* provides information on lipoprotein subclasses

2. *Window 2 (LMWH window)* provides information on several low molecular weight metabolites including amino acids, creatinine and ketone bodies.
3. *Window 3 (LIPID window)* is applied to a serum lipid extract therefore measuring available serum lipid constituents and fatty acid saturation including sphingomyelins.

The metabolomics samples were quality controlled by Brainshake Ltd®. Abnormal blood samples were marked as;

1. *High pyruvate/ high lactate.* Whole blood kept at room temperature during sample collection procedure allows continued metabolism of glucose to pyruvate/ lactate. These were thought to be study-specific issues, potentially influencing the absolute metabolite concentrations.
2. *Low protein content.* Samples were tagged when comparisons were made to the distribution of albumin concentrations within large populations. Low protein content was thought to arise from potential dilution of samples.
3. *High ethanol based.* Arise from contaminations with the blood donation/ collection procedure therefore preventing quantification of glycerol and beta-hydroxybutyrate. High levels of ethanol may also artificially increase acetate concentrations.

2.5.5.2 Cord blood metabolome

The serum cord blood metabolome was analysed at Ludwig Maximilian, University of Munich using MS. At the time of analysis, there was no validated methodology for analysing the cord blood using NMR. MS is suitable for almost all specimen types and is associated with high sensitivity and therefore able to detect subtle changes in fetal exposures at the time of birth. Although it is acknowledged that MS can be associated with low reproducibility, the spectra provide relative concentrations of metabolites against a

known maternal standard; providing further detailed insight into the subtle changes of fetal exposures in offspring born to obese mothers.

Cord blood samples with a minimum volume of at least 150 µl were sent to the University of Munich on dry ice and immediately stored at -80°C. 50 µL of plasma were thawed and diluted with 450 µl methanol, containing internal standards representing different groups of metabolites (Harder *et al.*, 2011; Hellmuth *et al.*, 2012). After centrifugation, the supernatants were separated to enable the quantification of polar lipids, acylcarnitines, NEFAs and amino acids. Centrifugation was undertaken to obtain supernatants which were divided using the following techniques;

1. *Polar lipids.* Quantification by flow-injection mass spectrometry was used to analyse polar lipids. Samples were analysed with a triple quadrupole mass spectrometer (QTRAP4000, Sciex, Darmstadt, Germany) with an electrospray ionization source. The analysis comprised diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, sphingomyelins, lysophosphatidylcholine and sum of hexoses. However, the analytical technique applied here was not capable of determining the position of the double bonds and the distribution of carbon atoms between fatty acid side chains. The nomenclature for polar lipids are described as:

$CX:Y$

X is the length of the carbon chain

Y is the number of double bonds

OH indicates the presence of a hydroxyl group

a indicates an acyl chain bounded by an ester bond

e indicates an acyl chain bounded by an ether bond

2. *Carnitines.* Flow-injection mass spectrometry was used to analyse acylcarnitines. Samples were analysed with a triple quadrupole mass spectrometer (QTRAP4000, Sciex, Darmstadt, Germany) with an electrospray ionisation source.

3. *Amino acids.* Amino acid butylesters were determined by ion-pair liquid chromatography coupled with mass spectrometry detection (Bradfield *et al.*, 2012). Mass spectroscopy detection was performed with an API 2000 triple quadrupole instrument (Sciex, Darmstadt, Germany) with an APCI source operating in a positive ion ionization mode.

4. *NEFAs.* An ultra-performance liquid chromatography diphenyl column (Pursuit UPS Diphenyl, Varian, Darmstadt, Germany) was used for chromatographic separation with high performance liquid chromatography system (Bouchard, 2009). A hybrid triple quadrupole mass spectrometer, coupled to a high-performance liquid chromatography system was used for the identification of NEFAs. Fatty acids are separated according to chain length and number of double bonds. Similar to polar lipids, NEFAs are described as:

$CX:Y$

X is the length of the carbon chain

Y is the number of double bonds

Intra-assay quality control of each metabolite was defined as coefficient of variation of <30% within a single batch in comparison to control samples.

2.6 Data management

2.6.1 Data management for clinical, anthropometric and lifestyle data

Continuous data including anthropometric data were checked by assessing normal probability plots and histograms to identify potential outliers. Outliers were defined $\pm \geq 4$ standard deviations from the mean as well as from the previous measurement. Scatter plots were used to compare follow-up measures to baseline values. Measurements that appear outside the main distribution when plotted on normal probability plots and histograms were

deemed 'normal' if consistency was shown within scatter plots. Potential outliers were checked for data entry and measurement errors. If an anthropometric measurement for example was thought to be a 'true' outlier, the observation was dropped from the analysis. Variables generated for example total GWG and SSFT, were recalculated excluding the dropped observation to ensure consistency of the data.

2.6.2 Data management of biochemical variables

Normal probability plots and histograms were used to determine appropriate transformations and to identify potential outliers. If the distribution of a variable was non-normal, transformations to a logarithmic base 2 were undertaken as it was thought that this would ease interpretability for non-specialists. Scatter plots of the transformed data were constructed to compare the follow-up measures to baseline. For the biochemical analysis, outliers were defined as ≥ 3 standard deviations from the line of best fit on the scatter plot.

Outliers were assessed for measurement problems. Measurements considered outliers or biochemical values above or below levels outside the level of calibration range, a new variable was derived marking the value as an implausible value.

2.6.3 Metabolomics data management

Similar data management procedures were undertaken for both the maternal and cord blood metabolome. Metabolites were dropped if $\geq 70\%$ values were unmeasurable or missing. Assessment was made for the variation of metabolite distribution by gestational age at sample collection by assessing the R^2 and scatter plots as shown in Figure 2.6. If significant variation (defined as $R^2 \geq 0.1$) was present, metabolites were adjusted for gestational age at sample collection accordingly.

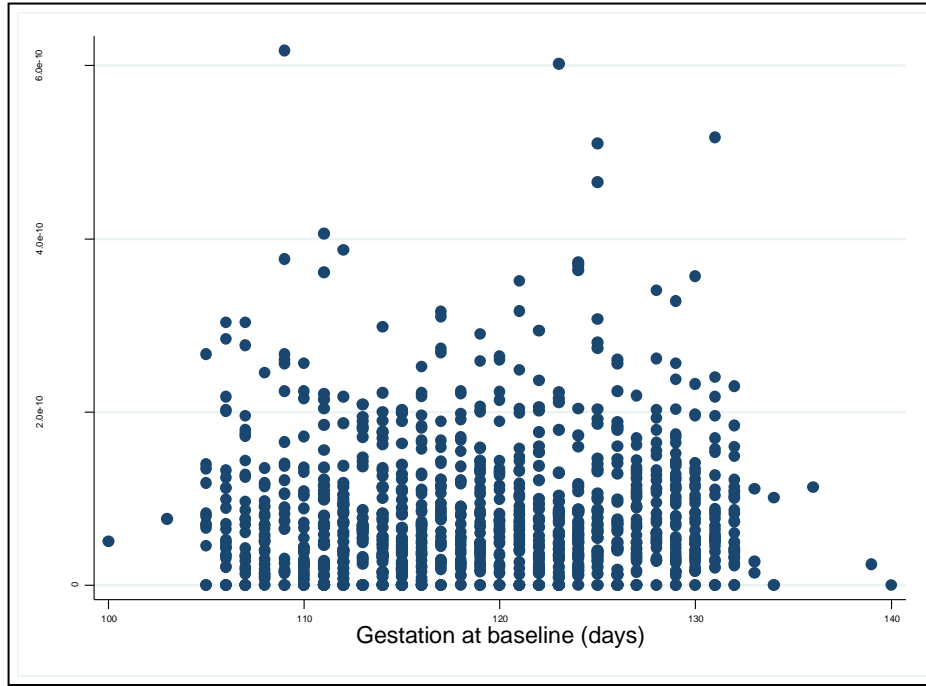


Figure 2.6: An example of a scatter plot for the maternal metabolite XXLVDLP as demonstration of data management techniques undertaken.

Variation was assessed with increasing gestational age of sample collection at each time point of biochemical collection, for maternal and offspring samples.

Statistical definitions of outliers were derived based on the number of samples analysed at each pregnancy time-point. An assumption was made that a biochemical outlier within a normal population has a 1 in 20 chance of occurring; therefore 1 in 40 chance of occurring below the level of detection and 1 in 40 chance of occurring above the level of detection.

The following formula was used to calculate the number of standard deviations an outlier was defined as, at each time point of the study using the following formula:

$$\text{invnorm}\left(\frac{1}{\frac{NS}{40}}\right)$$

where invnorm is the inverse of the cumulative of the normal distribution function and NS is the number samples analysed at each time point.

This methodology was used to calculate the definition of an outlier at each gestation in pregnancy;

1. *15-18⁺6 weeks' gestation*: 1083 samples underwent targeted NMR metabolomics therefore an outlier was defined as ± 4.074 standard deviations (SDs).
2. *27-28⁺6 weeks' follow-up visit*: 977 samples underwent targeted NMR metabolomics therefore an outlier was defined as ± 4.050 SDs
3. Cord metabolome: 607 samples underwent mass spectroscopy metabolomics analysis therefore an outlier was defined as ± 3.937 SDs

Box plots and scatter graphs were used to detect potential outliers using the original and transformed variable and subsequently characterised as an outlier when present in both the original and transformed data (Figure 2.7).

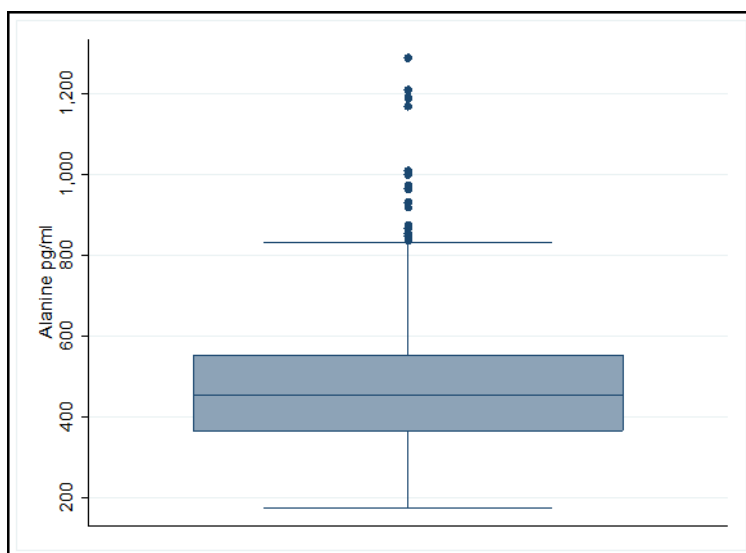


Figure 2.7: An example of a box plot for the cord blood metabolite alanine.

Plotted is the median, 25th and 75th percentiles for the cord metabolite.

Identified potential outliers were also checked for quality control issues (for example high pyruvate, ethanol, lactate). Outliers were assessed by 3 independent people and subsequently marked as an outlier within a new dataset. During analysis of the metabolomics data, sensitivity analysis was undertaken using a dataset with the outliers removed.

Transformation of the metabolomics data were checked using distributional plots (Figure 2.8). Where required, logarithmic transformation of the metabolites were undertaken. This method was undertaken to ensure that metabolites at the lower and upper levels of detection were not reported as missing values, as would occur if a logarithmic transformation was undertaken; thereby maintaining statistical power.

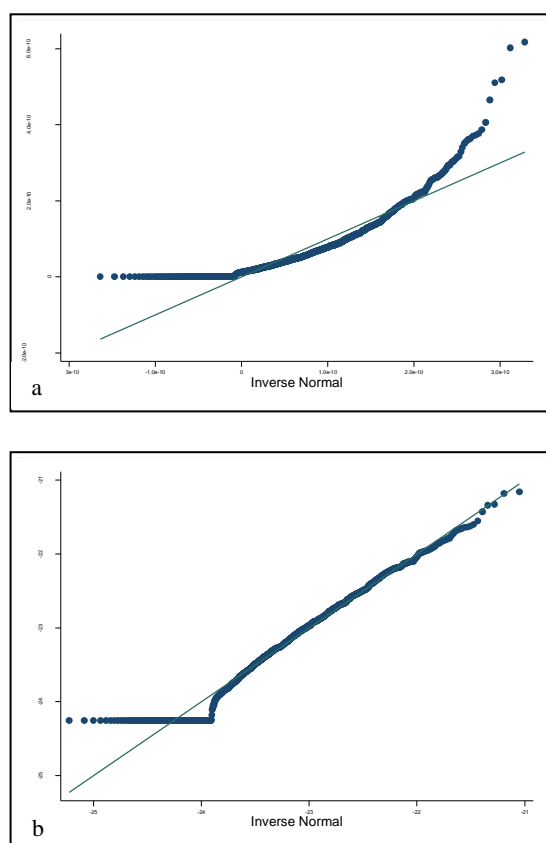


Figure 2.8: An example of the maternal metabolite XXLVDLP data management assessing for distributional issues before (a) and after (b) transformation.

2.7 Statistical analyses

2.7.1 Summary statistics

Binary and categorical variables were summarized using counts and percentages. The distribution of continuous variables was assessed using coefficients of skewness and then summarised by mean and standard deviation or median and interquartile range where appropriate.

2.7.2 Univariate analysis

Univariate analyses were undertaken if the outcome of interest was binary or categorical where the chi-square test was used. Mann-Whitney U tests or t-test were undertaken for continuous data dependent on the distribution. A 2-sided p-value with a statistical significance set as $p < 0.05$ was used throughout this thesis, for rejection of the null hypothesis.

2.7.3 Confounder selection

Confounders were selected a-priori with relevant clinical knowledge and the use of direct acyclic graphs. This method identifies potential confounders as variables along a biasing confounding pathway. Direct acyclic graphs select the smallest number of covariates to control for bias (also known as the minimal sufficient adjustment set). It should be noted, that due to the non-parametric nature, direct acyclic graphs are limited in their ability to describe many developmental programming hypothesis including interaction. Confounders with an explanation of selection are described in the individual chapters.

2.7.4 Multivariate analysis

Linear and logistic multivariate regression were used to estimate the relationship between exposures and continuous and binary outcomes respectively with adjustment for confounders. Regression analyses provide an equation of the best straight line through the observed data:

$$y = a + bx$$

where y is the outcome of interest, a is the intercept, b is the gradient of the line and x is the exposure variable. The gradient of the line, also known as the regression coefficient provides that change in the outcome for each unit change of the exposure. Calculations are based on the least squares method which is based on minimizing the differences between the observed and mean values.

Regression analyses assumes that the distribution of the residuals are normal, the relationship is linear and the standard deviation of the outcome is constant at all values of the exposure (i.e. no heteroscedasticity).

2.7.5 Interaction analysis

Interaction between two variables is defined as the presence of a varying association between the outcome of interest and the exposure, through an intermediary third variable.

An interaction term is specified as the following equation within a model;

$$y = a + bx + cx + (bx \times cx)$$

where y is the outcome of interest, a is the intercept, b is the gradient of the line, x is the exposure variable and c is a categorical/ continuous or binary confounder variable. The multiplicative term “bx × cx” is the interaction term which is pre-specified based on an a-priori knowledge added to the regression analyses. To assess if there is evidence of interaction, likelihood ratio tests were undertaken on nested models containing the interaction term as an additional factor.

2.7.6 Mediation analysis

Mediation analysis allows the investigation of an indirect relationship between an exposure and outcome, mediated through a 3rd variable. Potential mediators were identified using the

Baron Kenney Procedure which described a three-step method prior to undertaking a mediation analyses (Baron and Kenny, 1986);

1. Establishment of an exposure associated with the outcome
2. The exposure is a significant predictor of the proposed mediator variable (therefore if the mediator is not associated with the independent variable, there is no mediating role of the mediator).
3. The mediator is a significant predictor of the dependent variable.

Other statistical methods necessary to undertake causal mediation analyses for RCTs are described in the relevant chapters.

2.7.7 Missing data

Within the UPBEAT study missing data mostly resulted from consent withdrawal or attrition. The presence of missing data, has three important implications for the analyses of longitudinal data;

1. Under certain circumstances, missing data can introduce bias, potentially leading to misleading inferences.
2. Missing data in longitudinal studies tends to be spread sporadically over many subjects, and depending on how highly correlated the missing data is in comparison to the observed data, it may result in a loss of precision and power.
3. Missing data may be unbalanced between the two arms within a randomised controlled trial setting.

Statistical methods considering missing data require careful assumptions on the nature and pattern of missingness which if misunderstood may result in the introduction of significant biases. For this thesis, review of methods of data collection, preparation and analysis were undertaken to highlight the issues and mechanisms of missing data.

2.7.7.1 Mechanisms of missing data

Understanding the mechanisms of missing data is critical in understanding the influence of missing data on specific analysis and undertaking specific missing data techniques. The missing data mechanisms were first classified by Rubin as Missing at Random (MAR), Missing Completely at Random (MCAR) and Missing Not at Random (MNAR) (Rubin, 1976). MNAR assumes that this missingness is dependent on the unobserved data, therefore cannot be ignored. MAR refers to a situation where the probability of data being missing is not associated or dependent on the unobserved observations but shares relation to the observed data, i.e. the pattern of missing data is conditional on another variable. MCAR refers to data, where complete cases are representative of the original sample therefore associations based on these complete cases are thought to be applicable to the larger population (Little 1992).

2.7.7.2 Strategies to deal with missing data

Missing data is common within follow-up of RCTs and observational studies. Separate strategies for clinical and biochemical (including metabolomic data) were devised to deal with missing data including the use of complete case analysis.

2.7.7.2.1 Missing clinical data

Missing data following randomisation was assumed as lost to follow-up. A step-wise four point framework adapted from *White et al*, was devised to deal with incomplete observations, allowing the correct statistical method to be chosen and subsequently implemented (White *et al.*, 2011; Dziura *et al.*, 2013), as described below;

1. Perform a main analysis of all observed data that are valid under a plausible assumption about the missing data (i.e. the use of a complete case analysis).

2. Perform a sensitivity analyses to explore the influence of departures from assumptions made within the main analysis.
3. Account for all randomised participants, at least within sensitivity analyses using multiple imputation under the MAR assumption where feasible.

2.7.7.2.2 Missing data methods

Complete Case analysis

Complete case analyses are undertaken under the MCAR assumption. However, complete case analysis can result in loss of statistical power due to list-wise deletion of variables and may result in bias because of partial loss of data. Within longitudinal analyses groups with missing data are likely to have significantly different demographic characteristics in comparison to those with complete data. Inclusion of these differing variables within regression, can be included within the multivariate models to reduce potential biases (Graham & Donaldson 1993).

Multiple imputation

Within this thesis, multivariate imputation by chained equation was used as a sensitivity analysis when comparing results obtained from complete case analysis, where applicable. Imputation by chained equations models a plausible set of values reflecting the uncertainty regarding the correct value to impute (Rubin 1987). Imputation introduces an error based variation within the parameter estimates known as ‘between imputation error’, i.e. modelling the uncertainty associated with the missing values therefore reducing potential biases.

Multiple imputation is broken down into three stages defined by the predictors (Yuan et al, 2008)

1. The missing data are filled in n times (defined by the user) to generate complete data sets (the number of complete data sets defined by the user).
2. Complete data sets are analysed using standard procedures in each individual dataset.
3. Results from all imputed datasets are combined to estimate inference between the outcomes and exposures.

For this thesis, comprehensive imputation models were, selecting a-priori predictors of missingness to preserve the associations or relationships among variables. With regards to repeated measures, baseline values for both maternal and infant data were used within the model. Where multiple imputation was used, the dataset was replicated 50 times with 10 burn-in iterations.

2.7.8 Multiple testing

Performing many statistical tests, associations may be statistically significant purely by chance, resulting in many false positives. For example, if assessment was made of 200 maternal exposures on a chosen offspring outcome, based on $\alpha=0.05$, it is assumed that there will be 10 significant results. Statistical methods have been developed to account for the multiplicity of statistical testing. These methods are subcategorised into controlling the family-wise error rate and false discovery rate. The aims of both these statistical tests are to control the type 1 error, defined as incorrect rejection of the null hypothesis. When controlling for the family wise error rate or the false discovery rate, it is assumed that the individual tests are independent of one another and are not correlated within groups of comparisons. Within this thesis, correction for a false discovery rate using the Benjamin-Hochberg procedure was utilised as an approach to multiple testing.

2.7.8.1 False discovery rate

The false discovery rate (FDR) controls for significant results which are false positives in relation to the outcome. This methodology was originally devised by Benjamin & Hochberg and robustly controls for the type 1 error without loss of statistical power (Benjamini and Hochberg, 1995). This methodology ranks the p-values for a given number of statistical tests, and each individual p-value is compared to the critical p-value defined as:

$$\left(\frac{\text{rank}}{\text{total number of tests}} \right) \times \text{False discovery rate}$$

Therefore, the largest p- value was determined by;

$$p < \left(\frac{\text{rank}}{\text{total number of tests}} \right) \times \text{False discovery rate}$$

Those p-values smaller than this calculated value are statistically significant. It should be noted to report a false discovery p-value, generated p-values by the Benjamin and Hochberg methodology need to be transformed using the following equation;

$$\text{FDR adjusted pvalue} = \text{Minimum} \left(\frac{\text{original pvalues}}{\text{critical FDR pvalue}} \times 0.05 \right)$$

The Benjamin-Hochberg methodology is suitable for use when undertaking large number of comparisons, for example assessing the metabolome in relation to an outcome.

All statistical analysis were performed using Stata Version 14 and R (<https://www.r-project.org/>).

Chapter 3 Relation of clinical and metabolic characteristics to neonatal adiposity among obese pregnant women

3.1 Background

One in four women of childbearing age globally are obese (Ng *et al.*, 2014). As discussed in *Chapter 1: Introduction*, obesity represents a significant burden to antenatal healthcare in 21st century medicine, the predominant complication being a heightened risk of GDM (Kim *et al.*, 2014). Fetal macrosomia, associated with increased fetal and neonatal adiposity, is a major determinant of adverse pregnancy outcomes in obese women and has been independently associated with childhood and adolescent obesity (Metzger *et al.*, 2009; Huang *et al.*, 2011; Cunningham *et al.*, 2014; Sovio *et al.*, 2016).

A central role for fasting maternal glycaemia in fetal fat accretion is widely accepted, being exemplified by the HAPO study which identified a linear relationship between mild hyperglycaemia and neonatal adiposity in women without overt GDM (Metzger *et al.*, 2009; Farrar *et al.*, 2016a). As well as glucose, fatty acids, triglycerides and amino acids have also been implicated (Friedman, 2015; Scholtens *et al.*, 2016). High throughput metabolomics now enables quantification of a wider range of maternal metabolites to better characterise the determinants of neonatal adiposity, and this approach was recently adopted for samples obtained from a sub-group of HAPO women, at the time of the OGTT at 28 weeks' gestation. Scholtens *et al* reported that increased neonatal adiposity, assessed as SSFT, was associated with maternal clusters of acylcarnitines, ketone bodies, long and medium chain fatty acids when measured at one hour post glucose load at the time of the OGTT challenge, suggesting a possible mechanistic role for these metabolites (Scholtens *et al.*, 2016).

Amongst obese women, excessive fetal growth precedes the clinical diagnosis of GDM at 20 weeks' gestation (Sovio *et al.*, 2016) and metabolic dysfunction is evident in overweight

and obese women in early pregnancy, including a recent report identifying overt insulin resistance in 30% of women (<20 weeks gestation) (Harreiter *et al.*, 2016). It is currently unknown whether early pregnancy maternal exposures in obese women, including demographic and metabolic variables, contribute to the development of fetal adiposity. Precise characterisation of early pregnancy maternal risk factors for neonatal adiposity would offer the potential for development of targeted early interventions to prevent excessive fetal fat accretion in obese women (Sovio *et al.*, 2016).

The overall aim of this study was to examine the relationship between early (15-18 weeks' gestation) pregnancy maternal clinical and biochemical variables with neonatal adiposity as assessed by SSFT.

Using data from the UPBEAT trial, maternal demographic, clinical, anthropometric data together with biochemical markers previously associated with insulin resistance (including candidate biochemical markers and metabolome analysed by NMR, as discussed in *Chapter 2: Methods*) were assessed using multivariable regression. Late second trimester biochemical variables were assessed as potential mediators of early pregnancy maternal characteristics and neonatal adiposity using mediation analyses.

3.2 Research design and methods

3.2.1 Study design and population

The study was undertaken with data and samples from UPBEAT; a multicentre RCT assessing the effect of a behavioural lifestyle intervention in 1555 obese pregnant women (Poston *et al.*, 2015). Further details of the study participants including trial inclusion and exclusion criteria are provided in *Chapter 2: Methods*. As the incidence of GDM (IADPSG criteria), delivery of a LGA neonate and neonatal fat mass distribution did not differ between the intervention and control arms of the UPBEAT study (Poston *et al.*, 2015); the study was treated as a cohort for the purposes of this analysis. Live-born neonates with anthropometric measurements within 72 hours of birth were included in the analysis. Neonates with major congenital abnormalities were excluded.

3.2.2 Neonatal outcomes

The neonatal anthropometric outcomes of interest were continuous measures of adiposity assessed by SSFT (subscapular and triceps SFT combined). As secondary outcomes, individual SFT (subscapular and triceps) were assessed to determine the relative contribution of each, to the observed associations. Further details regarding measurement of neonatal anthropometric measures are provided in *Chapter 2: Methods*.

3.2.3 Maternal variables

Maternal early pregnancy sociodemographic, medical and family history were recorded at 15-18⁺⁶ weeks' gestation. Measures of maternal anthropometry including height, weight, waist, arm and thigh circumferences and 4 SFT (suprailiac, subscapular, triceps and biceps) were made at 15-18⁺⁶ and 24-28⁺⁶ weeks' gestation. Further details regarding collection of maternal anthropometric measurements are provided in *Chapter 2: Methods*. Non-fasting maternal blood samples were obtained at 15-18⁺⁶ weeks' gestation for evaluation of

seventeen candidate biochemical markers previously implicated with insulin resistance, together with quantification of 46 relevant metabolites related to amino acids, fatty acids, cholesterol, glycolysis and carbohydrates, all components of a targeted metabolome obtained by NMR spectroscopy (Wurtz *et al.*, 2016). Details of measurement of candidate biomarkers and metabolomics analytical methods are provided in *Chapter 2: Methods*.

At 24-28⁺⁶ weeks' gestation, all women underwent an OGTT. IADPSG criteria were used for GDM diagnosis (fasting venous glucose >5.1mmol/L, 1hour venous glucose \geq 10.0mmol/L and 2hour venous glucose \geq 8.5mmol/L). The candidate biochemical marker analyses at 24-28⁺⁶ weeks' were undertaken on fasting samples obtained at the OGTT visit.

3.2.4 Statistical analysis

Maternal and neonatal demographic and clinical characteristics were summarised using the mean or percentages as appropriate. Anthropometric measures were treated as continuous variables. Selection bias was assessed by comparing maternal and offspring characteristics between infants with and without neonatal anthropometric measurements. Data for all biochemical markers including those derived from targeted metabolomic analysis were transformed to a normal distribution where appropriate and assessed for linearity. Data management techniques including outlier detection and transformations are discussed in *Chapter 2: Methods*. Possible non-linearity of associations between maternal exposures and neonatal outcomes were tested by assessment of fractional polynomial statistics and with inspection of graphical plots.

3.2.4.1 Relationship with maternal demographic, anthropometric and biochemical variables at 15-18⁺⁶ and 24-28⁺⁶ weeks' gestation and neonatal adiposity

To assess the association between early pregnancy maternal exposures and continuous measures of neonatal adiposity (SSFT, subscapular and triceps SFT), univariate analysis were undertaken using linear regression. Significance tests are presented as unadjusted p-values, and with correction for multiple testing using FDR < 0.05 (Benjamini and Hochberg, 1995). Multivariate linear regression was then performed, adjusting for maternal confounders (described below). Confounders were determined using a-priori knowledge based on established associations, and direct acyclic graphs were drawn to identify potential sources of bias (Greenland *et al.*, 1999). Associations between maternal biochemical variables measured in fasting samples at 26-28 weeks' with neonatal adiposity were similarly investigated with adjustment for a-priori defined confounders (described below). Associations between neonatal sex and SSFT and individual SFT were also explored for evidence of interaction.

3.2.4.2 Confounder selection

As this study was an exploratory analysis, only environmental characteristics were considered as potential confounders. Maternal confounders adjusted for were; current smoking status in early pregnancy (smoker in early pregnancy vs. non-smoker/ ex-smoker), socioeconomic status (socioeconomic deprivation defined as 4th and 5th socioeconomic deprivation quintiles vs. no socioeconomic deprivation defined as 1st to 3rd socioeconomic deprivation quintiles) and educational attainment (>11 years in full time education vs. <11 years in full time education) as well as neonatal gestation at delivery (Model 1). Further adjustment was made for GDM (presence of GDM vs. no GDM) and GWG (assessed as net weight gain from early pregnancy to the end of pregnancy) (Poston *et al.*, 2015) (Model 2). GDM and GWG are associated with maternal biochemical profile at the time of the

OGTT as well as the development of neonatal adiposity (Rowan *et al.*, 2011; Scholtens *et al.*, 2016). Biochemical variables were further adjusted for maternal parity (nulliparous vs. multiparous) and ethnicity (reference population- white ethnicity).

3.2.4.3 Mediation analysis to assess the association between maternal variables collected in early and late 2nd trimester with neonatal adiposity

To assess whether maternal biochemical variables at 24-28⁺⁶ weeks' acted as intermediates in any observed associations between early pregnancy maternal variables and neonatal adiposity, mediation analysis with parametric regression was performed. This involved estimation of multivariate regression models; one with the mediator conditional on early pregnancy variables and covariates, and the second with neonatal adiposity, conditional with early maternal exposures and potential confounders (Hicks and Tingley, 2011) (Figure 3.1).

3.2.4.4 Sensitivity analysis

A sensitivity analysis was undertaken following exclusion of infants delivered pre-term (<37 weeks' gestation). A second sensitivity analysis was performed, removing observations with high leverage points (defined by Cook's Distance $D_i > 4/n$) (Altman and Krzywinski, 2016).

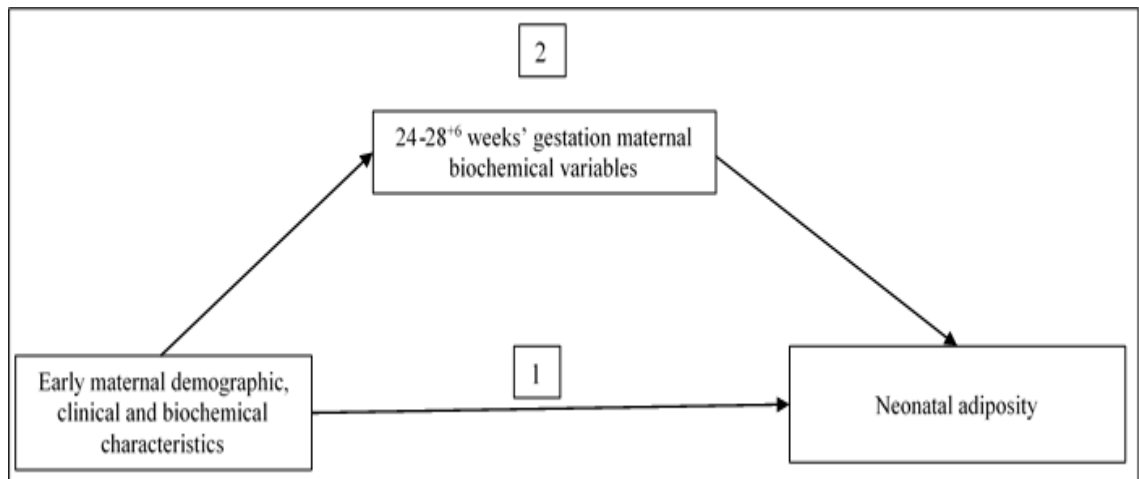


Figure 3.1: Diagrammatic illustration of mediation analysis.

Diagram to show multivariate regression analyses undertaken to establish whether greater neonatal adiposity associated with maternal obesity is the (1) direct influence of maternal characteristics in early pregnancy or (2) whether this is mediated through maternal biochemical pathways in late pregnancy as measured at the time of oral glucose tolerance test (26-28 weeks' gestation).

3.3 Results

3.3.1 Demography

Of the 1522 live-born neonates, 502 (32.3%) had anthropometric measurements within 72 hours of birth (Figure 3.2). Their mother's median BMI was 35.5kg/m² (range 32.9 to 38.9). 30.1% of women developed GDM, with dietary advice being the most common mode of treatment (11.6% of the total cohort) (Table 3.1). The median (IQR) gestational age at delivery for the neonates was 39.8 (38.7 to 40.9) weeks and mean (SD) birthweight was 3.49kg (0.47). Table 3.1 shows mean values for SSFT, abdominal and mid-upper arm circumferences.

Compared with the 1019 neonates not followed up at birth, there were no differences in maternal age, socioeconomic deprivation, BMI or incidence of GDM in comparison to those studied (Table 3.2). There were fewer neonates born to black mothers among those with anthropometric measures compared to those without (Table 3.3). Those included in this study had higher gestational age at delivery and neonatal birthweight (Table 3.3). Non-linear regression (fractional polynomials) statistics and scatter plots were used, but these did not suggest the presence of non-linear associations between continuous predictors and measures of neonatal adiposity. Following assessment of the distribution of neonatal SSFT using kernel density plots and interaction analyses; there was no evidence of moderation by neonatal sex (Figure 3.3). Multivariate analyses were adjusted for offspring sex and not further stratified by neonatal sex.

3.3.2 *Associations between maternal exposures assessed at 15-18⁺⁶ weeks' gestation and neonatal adiposity*

Data from univariate analysis is summarised in Figure 3.4.

3.3.2.1 Maternal demography

Following multivariate analyses, multiparity was the strongest determinant of neonatal adiposity as assessed by SSFT, being positively associated with an increase of 1.06mm (0.58 to 1.53; $p<0.001$) compared to nulliparous women (Model 1 and 2; Table 3.4). Each 1 kg increase in maternal birthweight was associated with a 0.55 mm increase in neonatal SSFT (95% CI 0.58 to 1.53; FDR adjusted p -value= 0.04) independent of GDM, total GWG, gestation at delivery and offspring sex (Model 1 and 2; Table 3.4). Similarly, maternal parity and maternal birthweight (per 1kg increase) were positively associated with neonatal subscapular SFT (multiparous vs. nulliparous, 0.45mm; 0.19 to 0.71; $p=0.01$; 0.34 mm; 0.11 to 0.58; $p=0.02$ respectively) (Table 3.5). Maternal parity was also positively associated with neonatal triceps SFT (0.61; 0.33 to 0.88; $p<0.001$) (Table 3.5). Maternal Black ethnicity was associated with lower neonatal SSFT after adjustment for maternal confounders, gestation at delivery and offspring sex (Model 1) but not after adjustment for GDM and GWG (Model 2) (Table 3.4).

3.3.2.2 Maternal anthropometry, 15-18 week's gestation

Following multivariate analyses, maternal triceps SFT at 15-18 weeks' was positively associated and suprailiac SFT negatively associated with neonatal SSFT; 0.04mm per 1mm (0.01 to 0.07; FDR adjusted $p=0.02$); -0.04mm per 1mm (-0.06 to -0.01; FDR adjusted $p=0.01$), respectively (Model 1 and 2; Table 3.4). Similarly, maternal triceps SFT (per 1mm) was positively associated with neonatal triceps SFT (0.02; 0.01 to 0.04; $p=0.02$) and maternal suprailiac SFT (per 1mm) was negatively associated with neonatal triceps SFT (-0.02mm; -0.03 to -0.01; $p=0.01$) (Table 3.5).

3.3.2.3 Maternal biochemical variables, 15-18 weeks' gestation.

Independent of maternal GDM, total GWG, socioeconomic deprivation, educational attainment and early pregnancy smoking, parity and ethnicity, higher vitamin D, serum glutamine, ketone bodies (serum acetate, acetoacetate and 3-hydroxybutyrate) and serum glycoprotein acetyls were positively associated with higher neonatal SSFT prior to correction for multiple testing (Models 1 and 2; Table 3.4). Serum cholesterol, aspartate transaminases and alkaline phosphatase were associated with a decrease in neonatal SSFT independent of maternal confounding but none remained significant after correction for FDR (Models 1 and 2).

3.3.3 *Association between maternal metabolic profile assessed in late 2nd trimester and neonatal adiposity*

Univariate analyses are summarised in Figure 3.5. Following multivariate analyses, maternal fasting serum glucose, insulin, C-peptide and glycoprotein acetyls, were positively associated with increasing neonatal SSFT following adjustment for confounders and FDR (Model 1 and 2; Table 3.6). Although metabolites including lactate, pyruvate and amino acids (including leucine, valine, tyrosine) were positively associated with neonatal SSFT, these were no longer significant following correction for multiple testing (Models 1 and 2). Maternal serum cholesterol and valine were negatively associated with neonatal SSFT (Model 1 and 2), but no longer significant following correction for multiple testing (Model 2, Table 3.6). Maternal fasting glucose, serum C-peptide and insulin at 24-28⁺⁶ weeks' gestation, were positively associated with neonatal subscapular SFT, whereas none of the 153 biochemical measures assessed at 24-28⁺⁶ weeks' were associated with neonatal triceps SFT (Table 3.7).

3.3.4 Inter-relationships between early pregnancy maternal characteristics, late 2nd trimester maternal metabolic profile and neonatal adiposity

Mediation analyses identified an indirect partial mediation of the relationship between maternal parity, fasting glucose at 24-28⁺⁶ weeks' and neonatal adiposity (multiparous vs. nulliparous; indirect effect 0.05; 0.01 to 0.03; p=0.01) (Table 3.8). Similarly fasting glucose partially mediated the relationship between increasing maternal triceps SFT at 15-18⁺⁶ weeks' gestation and neonatal SSFT (per 1mm increase; indirect effect 0.01; 0.00 to 0.01; p=0.04) but no biochemical mediator was identified for the observed association with maternal birthweight and neonatal SSFT (Table 3.8). In contrast an inverse, partial indirect effect was observed between maternal suprailiac SFT, fasting glucose and neonatal SSFT (per 1mm increase; indirect effect -0.01; -0.01 to 0.00; p=0.04) (Table 3.8). For the remaining biochemical markers (insulin, C-peptide and glycoproteins acetyls) which at 24-28⁺⁶ weeks' gestation were associated with neonatal SSFT, there was no evidence of an indirect mediatory role with early maternal pregnancy variables.

3.3.5 Sensitivity analysis

Exclusion of pre-term births (n=20) and removal of observations demonstrating significant leverage showed (n=23) no differences in the observed associations (Figure 3.6).

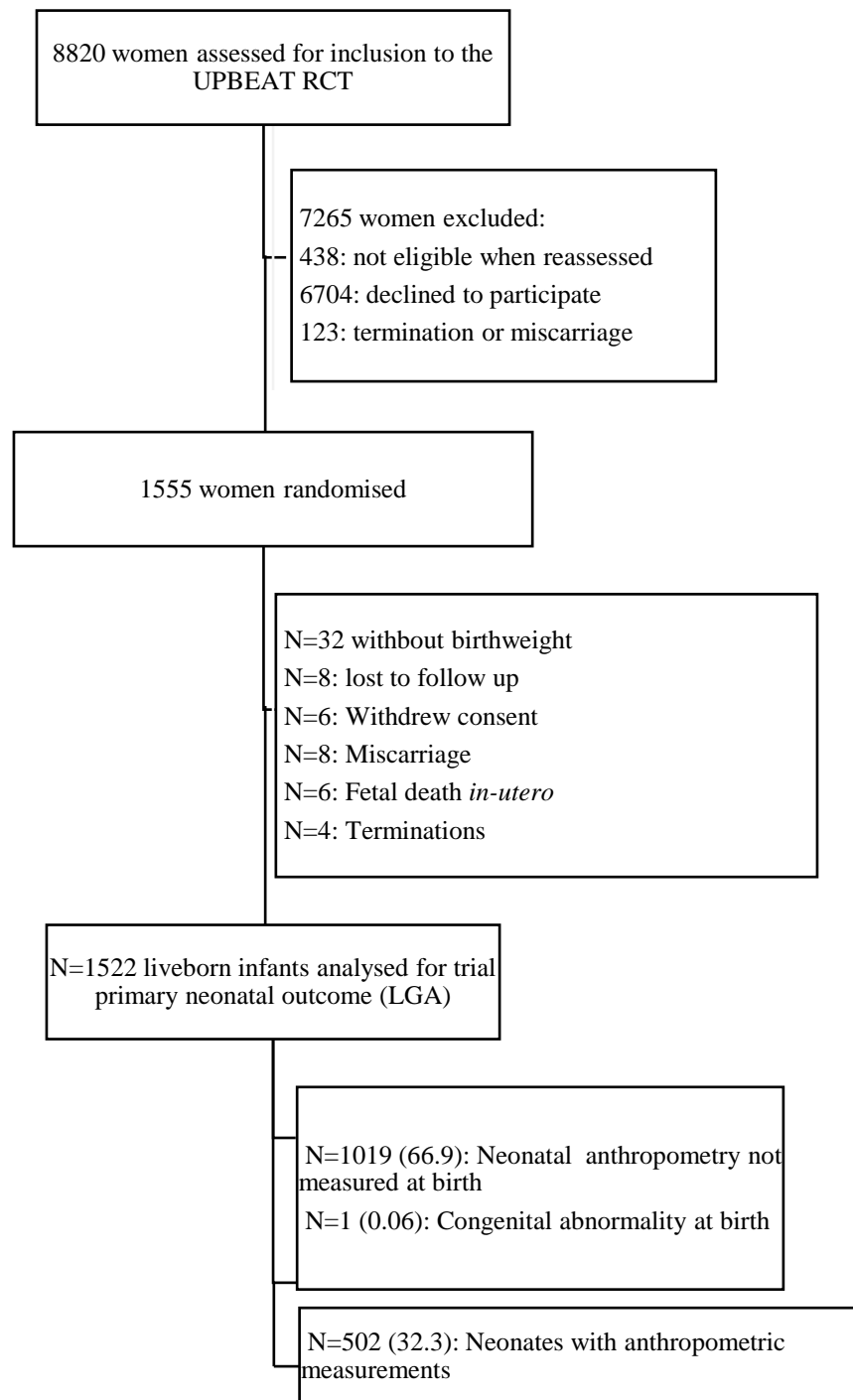


Figure 3.2: Flow diagram of neonates with anthropometric measures included in this analysis.

Abbreviations; LGA- Large for Gestational Age infants; RCT- Randomised Controlled Trial; UPBEAT- UK Pregnancies Better Eating and Physical Activity Trial.

Maternal		Mean (SD)/Median (Range)/N (%)
		N=502
Clinical history		
Age (years)		30.9 (5.5)
BMI (kg/m ²)		35.5 (32.9 to 38.9)
Ethnicity	White	344 (68.5)
	Black	108 (21.5)
	Asian	25 (5.0)
	Other	25 (5.0)
Time spent in full time education <12 years		55(10.9)
Socioeconomic deprivation		393 (78.6)
Multiparous		246 (49.0)
Smoker in early pregnancy		29 (5.8)
Clinical examination		
Systolic blood pressure (mmHg)		N=495 119 (11)
Sum of skinfolds (mm)		N=496 124.0 (26.0)
Upper midarm circumference (cm)		N=497 37.0 (4.0)
Antenatal history		
GDM		149 (29.6)
GWG (kg)		7.52 (4.2)
Treatment for GDM		
Insulin, diet and metformin		N=149 32 (21.5)
Metformin and diet		N=149 35 (23.5)
Diet only		N=149 56 (37.6)
None/Other		N=149 26 (17.4)
Neonatal		
Gestational age at delivery		39.9 (38.7 to 40.9)
Birthweight (kg)		3.5 (0.47)
Sum of skinfold thicknesses (mm)		11.0 (2.6)
Upper midarm circumference (cm)		11.5 (1.0)
Subscapular skinfold thickness (mm)		5.7 (1.4)
Triceps skinfold thickness (mm)		5.3 (1.5)

Table 3.1: Demographic and clinical data of maternal-neonate pairs included within this analysis (n=502).

Abbreviations; BMI- Body Mass Index; GDM- Gestational Diabetes; GWG- Total gestational weight gain

Maternal		Neonatal anthropometry		No neonatal anthropometry		p-value
		N	Mean (SD)/N (%)	N	Mean (SD)/N (%)	
Maternal demographics						
Maternal age (years)		N=502	309 (5.5)	N=1052	30.30 (5.53)	0.06
Maternal BMI (kg/m ²)		N=502	36.6 (5.0)	N=1052	36.14 (4.63)	0.07
Maternal ethnicity	White	N=502	344 (68.5)	N=1052	629 (6.7)	0.20
	Black		108 (21.5)		293 (27.9)	0.01
	Asian		25 (5.0)		70 (6.7)	<0.001
	Other		25 (5.0)		60 (5.7)	0.56
Multiparity		N=502	246 (49.0)	N=1052	634 (60.3)	<0.001
Current smoker		N=502	29 (5.8)	N=1052	79 (7.5)	0.21
IMD quintiles	Least deprived 1	N=500	24 (4.8)	N=1048	41 (3.9)	0.30
	2		37 (7.4)		66 (6.3)	
	3		46 (9.2)		131 (12.5)	
	4		169 (33.8)		364 (34.7)	
	Most deprived 5		224 (44.8)		446 (42.6)	
Pre-pregnancy Maternal anthropometry						
Triceps (mm)		N=498	32.7 (8.3)	N=1036	33.4 (9.5)	0.14
Biceps (mm)		N=498	21.5 (7.1)	N=1040	22.3 (8.4)	0.06
Subscapular (mm)		N=497	36.3 (10.3)	N=1041	35.0 (10.0)	0.02
Suprailiac (mm)		N=497	33.3 (10.9)	N=1040	31.5 (11.0)	<0.001
Sum of skin folds (mm)		N=496	123.9 (26.3)	N=1038	122.2 (28.1)	0.23
Antenatal clinical history						
GDM		N=494	138 (27.9)	N=811	200 (24.7)	0.19
PET		N=499	19 (3.8)	N=1008	35 (3.5)	0.74
Total GWG (kg)		N=465	7.5 (4.2)	N=628	7.5 (4.8)	0.92

Table 3.2: Comparison of maternal demography, anthropometry and antenatal clinical history for neonates with and without anthropometric measurements (included n=502, excluded n=1052).

Abbreviations; BMI-Body Mass Index; IMD-Index of Multiple Deprivation; GDM-Gestational diabetes; GWG-Gestational weight gain; PET-Pre-eclampsia.

Neonatal outcomes	Neonatal anthropometry N=502	No neonatal anthropometry N=1018	p-value
	Mean (SD)/N (%)	Mean (SD)/N (%)	
Birthweight (grams)	3.5 (0.5)	3.4 (0.7)	<0.01
Birthweight >4kg	609 (13.7)	141(13.9)	0.96
Birthweight <2.5kg	8 (1.6)	66 (6.5)	<0.001
Birthweight <1.5kg	0 (0.0)	21 (2.1)	.
LGA >90 th	47 (9.4)	86 (8.4)	0.52
LGA >95 th	22 (4.4)	48 (4.7)	0.77
SGA <10 th	56 (11.2)	120 (11.8)	0.72
SGA <5 th	24 (4.8)	61 (6.0)	0.34
Gestation at birth (weeks)	39.8 (1.5)	39.4 (2.6)	<0.001
Delivery <34 weeks	1 (0.2)	35 (3.4)	<0.01
Delivery <37 weeks	20 (4.0)	79 (7.8)	<0.01
Admission to NICU	14 (2.8)	108 (10.6)	<0.001

Table 3.3: Comparison between offspring birth characteristics of neonates with and without anthropometric measurements (included n=502, excluded n=1018).

Abbreviations; LGA-Large for Gestational Age; NICU- Neonatal Intensive Care Unit; SGA-Small for gestational age.

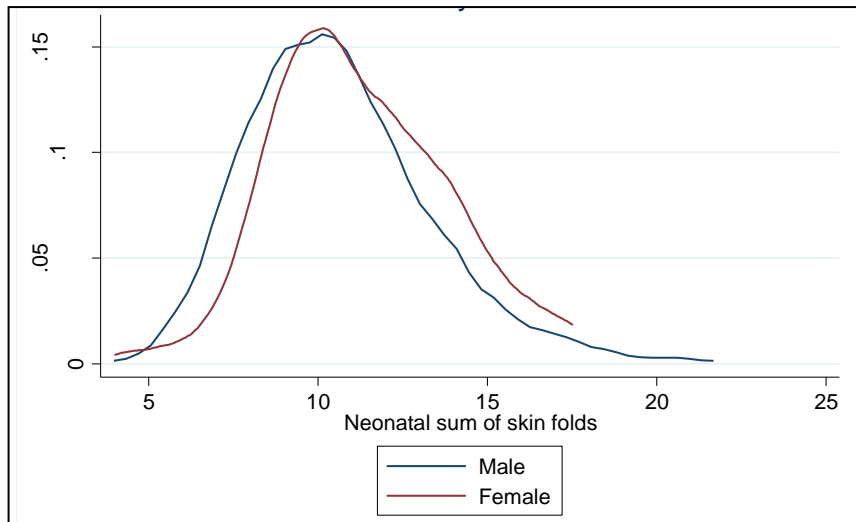


Figure 3.3: Distribution of neonatal sum of skinfold thicknesses stratified by offspring sex using Kernel density plots.



Figure 3.4: Univariate analysis of maternal demography, anthropometry and biomarkers measured at 15-18⁺6 weeks' gestation and neonatal adiposity assessed as sum of skinfold thickness, in offspring born to obese women (n=502).

	Model 1			Model 2			
	β	95% CI	P-value	β	95% CI	P-value	FDR adjusted p-value
Demographic & Clinical							
Black ethnicity	-0.79	-1.35 to -0.22	0.01	-0.46	-1.06 to 0.139	0.13	0.25
Multiparous	0.82	0.36 to 1.28	<0.001	1.06	0.58 to 1.53	<0.001	<0.001*
Maternal Birthweight (kg)	0.58	0.15 to 1.01	0.01	0.55	0.13 to 1.02	0.01	0.04*
Triceps SFT (mm)	0.04	0.01 to 0.07	0.01	0.04	0.01 to 0.07	<0.01	0.02*
Suprailiac SFT (mm)	-0.04	-0.06 to -0.02	<0.001	-0.04	-0.06 to -0.01	<0.01	0.01*
Biochemistry							
(log ₂) Cholesterol (mmol/L)	-1.56	-2.57 to -0.55	0.01	-1.44	-2.46 to -0.43	0.05	0.42
(log ₂) AST (U/L)	-0.77	-1.29 to -0.26	<0.01	-0.61	-1.15 to -0.07	0.03	0.57
(log ₂) ALT (U/L)	-0.39	-0.73 to -0.06	0.02	-0.36	-0.70 to -0.02	0.04	0.59
(log ₂) Fructosamine (μ mol/L)	-1.74	-3.30 to -0.18	0.02	-1.60	-3.17 to -0.02	0.05	0.65
(log ₂) Vitamin D	0.42	0.13 to 0.72	0.01	0.40	0.10 to 0.70	0.01	0.48
Metabolome							
Glutamine (mmol/L)	6.91	1.69 to 12.13	<0.01	6.72	1.34 to 12.10	0.02	0.37
Acetate (mmol/L)	0.77	0.23 to 1.32	0.01	0.71	0.15 to 1.27	0.01	0.50
Acetoacetate (mmol/L)	0.79	0.22 to 1.36	0.01	0.73	0.15 to 1.32	0.01	0.42
Glycoprotein acetyls (mmol/L)	2.61	0.54 to 4.67	0.01	3.04	0.91 to 5.17	0.01	0.82

Table 3.4: Association of early 2nd trimester maternal demography, clinical, anthropometry and biochemical factors with neonatal adiposity assessed as sum of skinfold thicknesses, in offspring born to obese women (n=502).

Results are presented as beta regression coefficients, 95% confidence intervals and p-value. Model 1- Adjustment made for maternal socioeconomic status, educational attainment and current smoking status, neonatal sex and gestation at delivery. Model 2- Adjustment made for 'Model 1', Gestational diabetes and total gestational weight gain. *Statistically significant at conventional $p < 0.05$ level, after correction for false discovery rate adjustment. Abbreviations; ALT-Alanine transaminase; AST- Aspartate transaminase; SFT-Skinfold thickness; 95% CI- 95% Confidence interval.

		Model 1				Model 2				
		β	Confidence Interval		p-value	β	Confidence interval		p-value	FDR corrected p-value
			LL	UL			LL	UL		
Neonatal subscapular thicknesses										
Multiparity		0.34	0.10	0.59	<0.01	0.45	0.19	0.71	<0.01	0.01
Maternal birthweight (grams)		0.00	0.00	0.00	<0.01	0.34	0.11	0.58	<0.01	0.02
Neonatal Triceps thicknesses										
Multiparity		0.49	0.23	0.75	<0.001	0.61	0.33	0.88	<0.001	<0.001
Skinfold thicknesses	Biceps (mm)	0.02	0.00	0.04	0.02	0.03	0.01	0.05	<0.01	0.02
	Triceps (mm)	0.02	0.00	0.04	0.01	0.02	0.01	0.04	<0.01	0.02
	Suprailiac (mm)	-0.02	-0.04	-0.01	<0.001	-0.02	-0.03	-0.01	<0.001	0.01

Table 3.5: Significant associations shown after multivariate analysis of maternal demography, anthropometry and biomarkers measured at 15-18⁺⁶ weeks' gestation and neonatal adiposity assessed as subscapular skinfold thickness and triceps skinfold thickness, in offspring born to obese women (n=502).

Model 1; Adjustment made for maternal socioeconomic status, early pregnancy smoking status, educational attainment, offspring sex and gestation at delivery; Model 2; Adjustment made for maternal socioeconomic status, early pregnancy smoking status, educational attainment, total gestational weight gain, gestational diabetes and offspring sex and gestation at delivery.

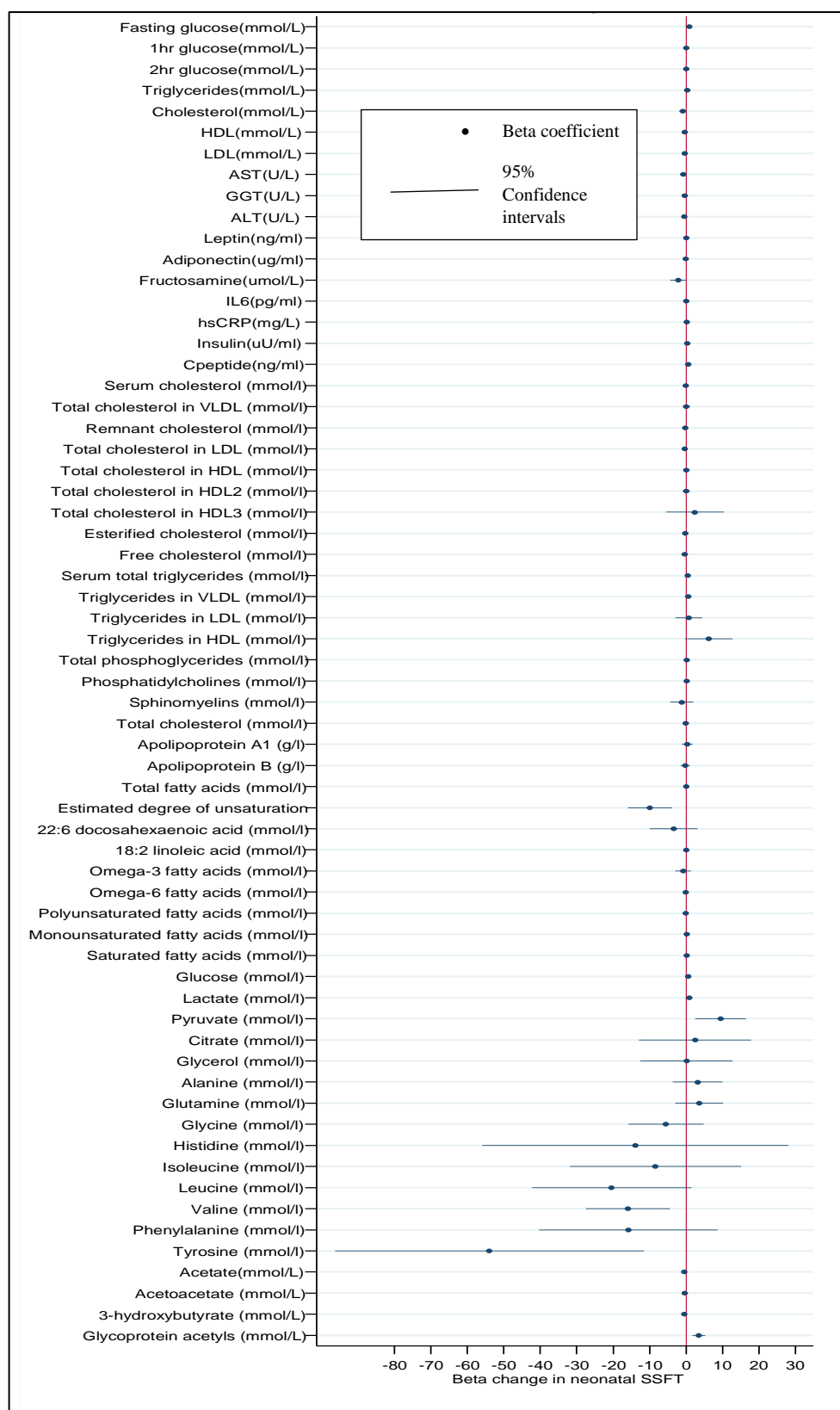


Figure 3.5: Univariate analysis of maternal biomarkers measured at 24-28⁺⁶ weeks' gestation and neonatal adiposity as assessed as sum of skinfold thicknesses in offspring born to obese women (n=502).

Abbreviations; ALT-Alanine transaminase; AST-Aspartate transaminase; CRP-C-Reactive protein; HDL-High density lipoprotein; GGT- Gamma-glutamyltransferase; LDL-Low density lipoprotein.

	Model 1			Model 2			
	β	95% CI	P-value	β	95% CI	P-value	FDR adjusted p-value
Biochemistry							
Fasting glucose (mmol/L)	0.86	0.42 to 1.30	<0.001	1.07	0.50 to 1.63	<0.001	0.02*
(log ₂) Cholesterol (mmol/L)	-1.05	-1.96 to -0.15	0.02	-1.17	-2.07 to -0.26	0.01	0.26
(log ₂) CRP (mg/L)	-0.70	-1.31 to -0.10	0.02	-0.55	-1.16 to 0.06	0.08	0.37
(log ₂) Insulin (μ U/ml)	0.34	0.14 to 0.54	<0.01	0.31	0.11 to 0.51	0.03	0.01*
(log ₂) C-peptide (mg/ml)	0.57	0.29 to 0.84	<0.001	0.50	0.23 to 0.78	<0.001	0.01*
Metabolome							
Lactate (mmol/L)	0.81	0.26 to 1.36	<0.01	0.62	0.06 to 1.18	<0.01	0.06
Pyruvate (mmol/L)	9.45	2.44 to 16.47	0.01	8.39	1.24 to 15.54	0.02	0.35
Leucine (mmol/L)	-23.01	-44.96 to -1.06	0.04	-26.15	-48.85 to -3.44	0.03	0.38
Valine (mmol/L)	-16.02	-27.46 to -4.57	0.01	-18.21	-29.86 to -6.57	<0.01	0.04
Tyrosine (mmol/L)	-51.60	-93.92 to -9.28	0.02	-51.83	-94.50 to -9.16	0.02	0.35
Acetate (mmol/L)	-0.51	-0.99 to -0.02	0.04	-0.49	-0.98 to -0.01	0.05	0.23
Glycoprotein acetyls (mmol/L)	3.31	1.50 to 5.12	<0.001	3.56	1.72 to 5.39	<0.001	0.01*

Table 3.6: Associations of late 2nd trimester maternal biochemical variables with neonatal adiposity assessed as sum of skinfold thicknesses, in offspring born to obese women (n=502).

Results are presented as beta regression coefficients, 95% confidence intervals and p-value. Model 1- Adjustment made for maternal socioeconomic status, educational attainment and current smoking status. Model 2- Adjustment for 'Model 1', Gestational diabetes and total gestational weight gain. * Significant at conventional $p < 0.05$ level, after false discovery rate adjustment. Abbreviations; CRP-C-Reactive protein; 95% CI- 95% Confidence interval.

	Model 1				Model 2				
	β	Confidence Interval		p-value	β	Confidence interval		p-value	FDR corrected p-value
		LL	UL			LL	UL		
Neonatal subscapular thickness									
Fasting glucose	0.59	0.35	0.83	<0.001	0.61	0.30	0.92	<0.001	0.01
(log ₂) Insulin (uU/ml)	0.20	0.09	0.31	<0.001	0.18	0.07	0.29	0.001	0.05
(log ₂) C-peptide (uU/ml)	0.31	0.16	0.46	<0.001	0.27	0.12	0.41	<0.01	0.02
Glycoprotein acetyls	1.95	0.99	2.91	<0.001	1.70	0.90	2.90	<0.001	0.01

Table 3.7: Significant associations following multivariate analysis of maternal biomarkers measured at 24-28⁺⁶ weeks' gestation and neonatal adiposity assessed as neonatal subscapular skinfold thickness and triceps skinfold thickness in offspring born to obese pregnant women (n=502).

Model 1; Adjustment made for maternal socioeconomic status, early pregnancy smoking status, educational attainment, offspring sex and gestation at delivery; Model 2; Adjustment made for maternal socioeconomic status, early pregnancy smoking status, educational attainment, total gestational weight gain, gestational diabetes, offspring sex and gestation at delivery. There were no significant associations between maternal biomarkers measured at 24-28⁺⁶ weeks' gestation and neonatal triceps skinfold thickness.

Pre-pregnancy and early 2 nd trimester maternal characteristics	Maternal biochemical variables measured at 24-28 ^{±6} weeks' gestation	Estimate *	95% CI		p-value
			LL	UL	
Multiparity	Fasting blood glucose (mmol/L)	0.05	0.01	0.03	0.01
	(log ₂) Insulin (μU/ml)	-0.02	-0.10	0.06	0.69
	(log ₂) C-peptide (ng/ml)	-0.07	-0.18	0.03	0.18
	Glycoprotein acetyls, mainly a1-acid glycoprotein	0.00	-0.02	0.02	0.85
Maternal birthweight (kg)	Fasting blood glucose (mmol/L)	0.01	-0.07	0.09	0.88
	(log ₂) Insulin (μU/ml)	-0.01	-0.07	0.05	0.72
	(log ₂) C-peptide (ng/ml)	0.01	-0.07	0.08	0.88
	Glycoprotein acetyls, mainly a1-acid glycoprotein	-0.00	-0.02	0.01	0.80
Maternal Triceps SFT (mm)	Fasting blood glucose (mmol/L)	0.01	0.00	0.01	0.04
	(log ₂) Insulin (μU/ml)	0.00	-0.00	0.01	0.13
	(log ₂) C-peptide (ng/ml)	0.00	-0.00	0.01	0.56
	Glycoprotein acetyls, mainly a1-acid glycoprotein	0.00	-0.00	0.00	0.73
Maternal suprailiac SFT (mm)	Fasting blood glucose (mmol/L)	-0.01	-0.01	0.00	0.04
	(log ₂) Insulin (μU/ml)	-0.00	-0.01	0.00	0.36
	(log ₂) C-peptide (ng/ml)	-0.00	-0.01	0.00	0.21
	Glycoprotein acetyls, mainly a1-acid glycoprotein	0.00	-0.00	0.00	0.70

Table 3.8: Indirect mediation analysis of early pregnancy characteristics, late 2nd trimester biochemical factors and neonatal adiposity, in offspring born to obese women (n=502).

* Adjusted for maternal socioeconomic status, current smoking in early pregnancy, gestational diabetes, total gestational weight gain, offspring sex and gestation at delivery. Abbreviations; LL- Lower Limit 95% Confidence interval; SFT- Sum of skinfold thickness; UL-Upper Limit 95% Confidence Interval.

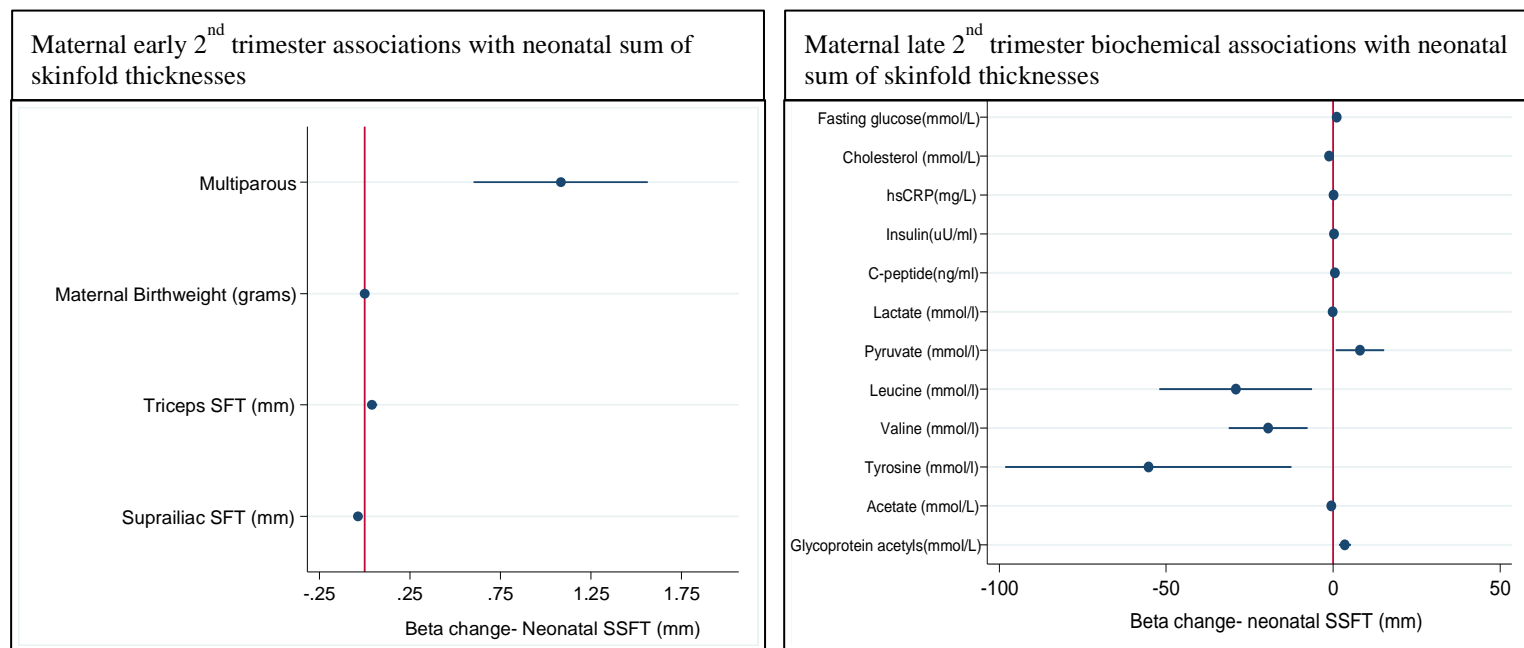


Figure 3.6: Sensitivity analyses following removal of pre-term deliveries (<37 weeks) and observations demonstrating high leverage (excluded n=43).

Abbreviations; ACE- serum acetate metabolite; Chol- plasma cholesterol; hsCRP- high sensitivity C-Reactive Protein; GP- serum glycoprotein acetyls; LA-serum lactate metabolite; Leu- serum leucine metabolite; Pyr- serum pyruvate metabolite; Suprailiac-maternal suprailiac skinfold thickness; Triceps- maternal triceps skinfold thicknesses; Tyr- serum tyrosine metabolite; Val- serum valine metabolite

3.4 Discussion

3.4.1 Summary of overall findings

- Modifiable maternal risk factors including early pregnancy (15-18 week' gestation) measures of maternal adiposity were linearly associated with measures of neonatal adiposity.
- An independent association was demonstrated with maternal birthweight and neonatal adiposity
- Maternal fasting glucose in the late second trimester was also found to partially mediate the effect of maternal parity and measures of early pregnancy adiposity with neonatal adiposity.

In this study the role of early and late second trimester pregnancy clinical and biochemical risk factors associated with neonatal adiposity, in offspring born to obese mothers was assessed, to better understand the mechanisms underlying increased offspring adiposity in early gestation. Increased neonatal adiposity is associated with greater adolescent obesity and insulin resistance (Huang *et al.*, 2011). Associations were investigated using a wider range of biochemical factors implicated with insulin resistance than previously explored (Metzger *et al.*, 2009; Harmon *et al.*, 2011; Friis *et al.*, 2013), including those determined within a targeted metabolome (Wurtz *et al.*, 2016). Using statistical modelling, key early pregnancy relationships of neonatal adiposity were identified, providing an improved understanding of early pregnancy risk factors for neonatal adiposity amongst obese women.

3.4.2 Early pregnancy maternal associations

The finding that maternal birthweight was associated with neonatal adiposity independent of gestational weight gain and gestational diabetes is, to our knowledge, novel. This relationship could potentially be explained by previously observed associations between high birthweight and maternal obesity mediated by maternal glycaemia which would be

anticipated, but was not observed (Sebire *et al.*, 2001). Population studies from Sweden have identified intergenerational associations of large for gestational age risk, which the authors suggest may be of genetic or epigenetic origin (Cnattingius *et al.*, 2012), although this may arise from strong intergenerational association of socioeconomic status, and associated lifestyle characteristics. Maternal birthweight may be a previously unrecognised and independent risk factor for neonatal adiposity which could contribute to early pregnancy risk assessment in clinical management.

The linear relationship between maternal parity and neonatal adiposity, concurs with observed associations of increasing maternal parity and higher birthweight (Wilcox *et al.*, 1996) and with one previous study describing an association of multiparity with neonatal adiposity, within a rural Indian population (Joshi *et al.*, 2005). These observations are likely to reflect cumulative weight retention associated with consecutive pregnancies (Rasmussen and Yatkine, 2009), an hypothesis which was supported by the observed partial mediation by fasting glucose within this study.

Previous reports of linear relationships between measures of maternal adiposity and neonatal sum of skinfold thicknesses were confirmed within this study, especially maternal peripheral fat as measured by triceps skinfold thickness, and increased neonatal adiposity at birth (Dube *et al.*, 2012). Triceps skinfold thickness in adults is commonly associated with an unfavourable metabolic profile (Björntorp, 1991; Jensen, 2008) and has been shown to track from childhood to adulthood (Santos *et al.*, 2016). Through the reported study, it has been demonstrated that increased maternal adiposity may influence this skinfold measurement in neonates predominately through maternal dysglycaemia.

The inverse relationship between a measure of maternal lower body fat, suprailiac skinfold thickness, and adiposity at birth may have a protective role as adipose tissue deposition

within the suprailiac region, has been associated with protection against cardiovascular disease as it is a recognised storage depot for dietary fat (Björntorp, 1991; Jensen, 2008). The unexpected lack of association with maternal BMI in this obese cohort and neonatal adiposity is likely to reflect the narrow range of maternal BMI and disparate body fat distribution (Farah *et al.*, 2011).

In assessment with previous findings, Black ethnicity was negatively associated prior to correction for multiple testing, with neonatal adiposity independent of maternal demographic characteristics, gestational diabetes and gestational weight gain. Although associations have been reported between Black ethnicity in relation to lower birthweight rather than measures of central adiposity (Singh and Yu, 1996; Okosun *et al.*, 2000), an association with reduced muscle rather than fat mass cannot be discounted within these reports. It may be relevant to longer term obesity risk that lower birthweight within Black ethnic groups, has been negatively associated with measures of adiposity in children between 5 and 10 years of age (Okosun *et al.*, 2000). Together, these observations suggest that Black ethnicity may have a protective effect on neonatal adiposity.

3.4.3 *Late 2nd trimester maternal metabolic profile associations with neonatal adiposity*

To our knowledge this is the most comprehensive analysis yet undertaken of biochemical markers of insulin resistance at the time of the oral glucose tolerance test and of relationships with neonatal adiposity. This data adds to previous reports that the permissive milieu leading to increased fetal fat accretion occurs before the diagnosis of gestational diabetes (Sovio *et al.*, 2016). Of the biochemical variables associated with dysglycaemia or insulin resistance measured in fasting maternal samples in late second trimester, four were positively associated with increasing neonatal adiposity, before and after adjustment for maternal gestational diabetes and gestational weight gain. Of these, three agree with a study from the HAPO cohort describing linear relationships between the associations with

maternal fasting glucose, raised insulin and C-peptide at the time of the oral glucose tolerance test with neonatal adiposity, as assessed by sum of skinfold thicknesses (Metzger *et al.*, 2009). A relevant study using Mendelian randomisation in up to 30,000 European origin mother-offspring pairs also supports a causal linear effect of greater maternal BMI and fasting glucose measured at the time of the oral glucose tolerance test with neonatal birthweight and ponderal index (Tyrrell *et al.*, 2016).

Glycoprotein acetyls, as identified by the metabolome; a known marker of inflammation, were positively associated with neonatal sum of skinfold thicknesses at 24-28⁺⁶ weeks' gestation. This novel finding may have relevance to a growing body of evidence that maternal markers of inflammation could contribute to persistently altered development of central pathways associated with satiety and appetite regulation in the offspring (Gaillard *et al.*, 2016).

Insulin resistance as assessed by fasting glucose, insulin and C-peptide at 24-28⁺⁶ weeks' gestation in obese pregnant women, was more strongly associated with neonatal subscapular than neonatal triceps skinfold thickness, and may have functional consequences as subscapular skinfold thickness, a measure of central body fat, with a lower measurement error compared to other skinfolds (Peiris *et al.*, 1988), is implicated in impaired glucose tolerance in children at 9-10 years of age, and with cardiovascular disease in adulthood (Srinivasan *et al.*, 2003; Freedman *et al.*, 2009).

As adiposity tracks through childhood (Huang *et al.*, 2011), the prevention of maternal dysglycaemia by pharmacological or lifestyle intervention has the potential to modify the life course trajectory of adiposity through early modification of central fat mass. However, two recent RCTs have reported no effect of early metformin treatment on the incidence of GDM or neonatal outcomes in a heterogeneous population of obese women (Chiswick *et*

al., 2015; Syngelaki *et al.*, 2016). This study implies that risk stratification of obese pregnant women e.g. by birthweight, parity, upper body fat adiposity or late second trimester glycaemia, would enable targeted pharmacological intervention in those at greatest risk, to achieve a meaningful reduction in dysglycaemia and subsequent neonatal adiposity.

Contrary to previous reports, there was no evidence for an association between early or late 2nd trimester triglycerides or non-esterified fatty acids concentrations and neonatal adiposity (32). This contrasts with findings from Catalano *et al.*, who proposed that a relationship between fatty acids and neonatal adiposity in women with gestational diabetes, may reflect a third trimester lipid rise rather than early pregnancy lipid metabolism (Catalano *et al.*, 2002; Schaefer-Graf *et al.*, 2008). This lack of association of triglycerides is consistent with the recent Mendelian randomisation study which found no evidence for a causal effect of triglycerides with neonatal birthweight or ponderal index (Tyrrell *et al.*, 2016).

3.4.4 *Strengths and limitations*

Strengths of this prospective longitudinal cohort study include, the measurement of a wide range of known candidate biochemical markers implicated with insulin resistance, including determinants of neonatal adiposity, enabling an a-priori data driven approach to assess the influence of early pregnancy *in-utero* exposures in obese pregnant women. This is one of the first exploratory studies assessing an extensive number of early maternal biochemical markers and high-throughput metabolomics of samples from pregnant obese women. Limitations include multiple testing to determine associations of early pregnancy maternal associations with neonatal adiposity, however correction for multiple testing using a false discovery rate was undertaken with application of a stringent threshold. However, findings require replication in independent cohorts. Neonatal anthropometric measures

were available in only a sub-group of the original cohort, but there were no differences in the maternal clinical characteristics and anthropometry between those without and with anthropometry at birth. The conclusions from the study are therefore potentially generalisable to other inner-city, socio-economically deprived populations. Maternal biochemical markers, in early pregnancy were assessed in non-fasting samples. Although measurement of fasting samples in early 2nd trimester may identify additional associations with neonatal adiposity, these would have limited clinical relevance for intervention where early pregnancy fasting samples are not collected within routine antenatal care. As this study was observational, causality cannot be assumed for the observed direct associations or mediation analyses (Richmond *et al.*, 2014).

3.4.5 Conclusion

In conclusion, further research is warranted to determine whether the associations reported here are likely to be causal. The study highlights the potential for targeted intervention in a stratified obese cohort including multiparous pregnant women, and those with excessive upper body fat deposition to successfully modify neonatal adiposity. This study also provides some support for early recognition and intervention of fasting dysglycaemia (Rowan *et al.*, 2011; Barker *et al.*, 2016) in obese pregnancies. Further randomised controlled trials are required to ascertain the potential influence of early risk stratification together with early pregnancy treatment for dysglycaemia within obese pregnancies to modify neonatal body composition.

Chapter 4 Cord blood metabolic profiles in obese pregnant women; insights into offspring growth and body composition.

4.1 Background

The increasing incidence of childhood obesity is a major public health concern. Recent global estimates from the WHO, suggest that 41 million children under the age of 5 years are overweight or obese (WHO, 2016). Observational cohort and experimental animal studies have strongly suggested that both the pre- and postnatal environments modulate developmental pathways that increase susceptibility to later obesity (Patel *et al.*, 2015). Offspring exposed to maternal obesity, excessive GWG and/or GDM *in-utero* are at an increased risk of obesity and altered glucose metabolism throughout the life-course (Lawlor *et al.*, 2011a; Fraser and Lawlor, 2014; Patel *et al.*, 2015; Sharp *et al.*, 2015). Exposure to maternal obesity *in-utero* is proposed to set the offspring on a trajectory of increased adiposity throughout life due to persistent changes in metabolic function (Giles *et al.*, 2015; Jin *et al.*, 2016). It is also suggested that altered fetal development is associated with exposure to excessive maternal nutrition *in-utero* influencing fetal developmental pathways leading to a persistent increased risk of obesity (Barker, 1997; Patel *et al.*, 2015).

Metabolomics has emerged as a tool which provides insight into the downstream products of transcriptional and translational processes as well as environmental exposures (Hivert *et al.*, 2015). This method enables the investigation of low-molecular weight molecules such as intermediate metabolites and signalling molecules which can be used as a tool to provide insight in the systemic perturbations of an individual as a result of pathophysiological *in-utero* exposure. Metabolites assessed in cord blood at delivery reflect fetal metabolic exposures during late gestation. However, few studies have assessed whether the cord blood metabolic profile could provide insight into mechanisms of offspring ‘programming’ as a result of early life maternal exposures.

Investigations of cord blood metabolic profiles have previously been conducted within small cases-control studies assessing associations with birth weight or postnatal growth velocities and with limited adjustment for *in-utero* confounding variables (Desert *et al.*, 2015). In a large birth cohort from Germany, certain cord blood metabolites were associated with birth weight (Taveras *et al.*, 2004). However, neonatal adiposity explains only 40% of the observed variation in birthweight, which may provide an improved assessment of fetal fat mass deposition *in-utero*.

The aim of this study was to assess whether a behavioural lifestyle intervention in obese pregnant women, focused on increasing physical activity, reducing dietary saturated fat and glycaemic load (Poston *et al.*, 2015), resulted in changes in the cord blood metabolic profile, including cord blood metabolome assessed using mass spectrometry and candidate biomarkers previously implicated with excess fetal growth and obesity in early life.

The secondary aim of the study, was to explore the relation between maternal antenatal characteristics including total GWG, early pregnancy BMI and GDM with the cord blood metabolic profile. As adiposity, has shown to track through childhood (Huang *et al.*, 2011; Giles *et al.*, 2015), further assessment was made for potential relationships between metabolites in the cord blood and measures of anthropometry in offspring at birth and at 6 months of age. Associations were also explored between cord blood metabolites and infant growth velocities between birth and 6 months.

4.2 Research design and Methods

4.2.1 Study Design

This study was a secondary analysis from the UPBEAT trial. To assess the primary aim of this study, the influence of the UPBEAT lifestyle intervention on the cord blood metabolic profile; the UPBEAT study was treated as an RCT (Briley *et al.*, 2014). As the secondary aim of the study was to assess the relationship of the cord blood metabolic profile with maternal clinical characteristics and neonatal and infant anthropometry, a cohort study design was chosen.

4.2.2 Study population

4.2.2.1 Primary aim

Women over the age of 16 years were recruited to the UPBEAT trial between 15-18⁺⁶ weeks' gestation, from inner-city populations with high socioeconomic deprivation. Detailed study design and protocol including inclusion and exclusion criteria are provided in *Chapter 2: Methods*.

4.2.2.2 Secondary aim

Mother-neonate pairs were included in the analyses if detailed neonatal anthropometric and cord blood metabolic data were available. Infants were included within a secondary analysis if they attended the follow-up appointment at 6 months of age and did not suffer from major ill health.

4.2.3 *Cord blood metabolic profile*

For this analyses, assessment of the cord blood metabolic profile was compromised of candidate biomarkers previously implicated with obesity and adverse growth together with cord blood metabolome quantified by MS.

4.2.3.1 Cord blood biomarkers

Candidate cord blood biomarkers assessed in this study include cord blood insulin, C-peptide, glucose, LDL-c, HDL-c, triglycerides, adiponectin, leptin, IGF I, II, IL-6 and TNF- α .

4.2.3.2 Metabolomic analyses

The cord blood metabolome comprised of lysophosphatidylcholines (LPCs), phosphatidylcholines, sphingomyelins, NEFAs, carnitines, tricarboxylic acid intermediates and amino acids.

Further details of methods of analyses for candidate biomarkers and metabolomic analyses in the cord blood are provided in *Chapter 2: Methods*.

4.2.4 *Maternal variables*

Maternal clinical characteristics investigated, included maternal early pregnancy BMI (kg/m^2); total GWG (kg) defined from pre-pregnancy to 34-36 weeks' gestation; GDM defined using the IADPSG's diagnostic criteria at 24-28⁺⁶ weeks' gestation as well as fasting glucose, 1 and 2 hour glucose concentrations at the time of the OGTT (Metzger *et al.*, 2010).

4.2.5 *Offspring anthropometry*

4.2.5.1 Neonate

Further details provided in *Chapter2: Methods*. Anthropometric measurements were made within 72 hours of birth by a trained midwife. Birthweight was recorded from maternal medical records and birthweight z-scores calculated (WHO, 2006; Briley *et al.*, 2014). Neonatal subscapular and triceps SFT were measured using Harpenden skinfold callipers in triplicate and SSFT were calculated (Briley *et al.*, 2014). Neonatal length was assessed using a neonatometer. Abdominal circumference was assessed at the level of the umbilicus and arm circumference measured midway between the elbow and shoulder bone (Briley *et al.*, 2014).

4.2.5.2 Infant

Further details provided in *Chapter2: Methods*. Anthropometric measurements were collected at 6 months of age by a trained midwife. Weight was assessed using SECA® scales, and length assessed in the supine position using an infantometer (Briley *et al.*, 2014). Triceps and subscapular SFT were measured in triplicate using Holtain callipers (Briley *et al.*, 2014). Midarm circumference was measured using the same methodology as the neonates. Where reference WHO population data were available, z-scores were calculated, adjusting for infant sex and age at measurement (WHO, 2006). At 6 months of age, these standards are applicable regardless of maternal ethnicity, socioeconomic status and mode of feeding (WHO, 2006; WHO and de Onis, 2006). The WHO definitions of catch-up and catch-down growth were used and specific definitions can be found in *Chapter 2: Methods* (WHO and de Onis, 2006).

4.2.6 Statistical analysis

4.2.6.1 Cord blood metabolic profile

Metabolites were standardized to mean metabolite concentrations and standard deviation over all eight batches for the purpose of this statistical analysis. Metabolites were included in the analyses if <70% data was missing. Cord blood biomarkers and metabolomic variables were assessed for normality and transformed appropriately. Dummy variables were generated for potential outliers defined as >4SDs, as discussed in *Chapter 2: Methods*. Variables were summarised using mean (SD) and median (IQR) where appropriate.

Principal component analysis was undertaken for the metabolomic data only, to reduce the number of metabolites based on a series of uncorrelated linear combinations of variables containing the most variance (Wold *et al.*, 1987). Following orthogonal rotation and identification of the number of clusters that best represented the data, metabolites with a loading ≥ 0.1 were considered to have a strong association with the cluster.

4.2.6.2 The effect of a UPBEAT lifestyle intervention on the cord blood metabolic profile

Assessment was made for any differences in maternal characteristics and birth outcomes between those included vs. those excluded from the analysis. Adjustment was made for any apparent differences in maternal characteristics at trial entry (15-18⁺⁶ weeks' gestation) between the two arms. The effect of the UPBEAT intervention were assessed using linear regression adjusting for minimisation variables used at trial randomisation (ethnicity, parity and maternal early pregnancy BMI).

4.2.6.3 Maternal associations with cord blood metabolic profile

Maternal antenatal variables (including early pregnancy BMI, GWG and GDM) were assessed in relation to the cord blood metabolic profile. To assess for potential relationships, multivariable linear regression was undertaken, where components of the cord blood metabolic profile were treated as the outcome and maternal antenatal variables as the exposure. Adjustment was made for offspring sex, gestational age at delivery and randomisation to the UPBEAT intervention.

4.2.6.4 Cord blood metabolic profile and offspring anthropometry

To assess the association between the cord blood metabolic profile (exposure) and subsequent offspring anthropometry (outcome) at birth, multivariable linear regression was used. Cord blood metabolic variables significantly associated with neonatal body composition were also assessed for potential relationships with infant anthropometry at 6 months of age, including early life growth velocities using multivariate linear and logistic regression, where appropriate.

Adjustment was made for confounders, selected a-priori based on clinical knowledge with the aid of direct acyclic graphs as summarised in, Table 4.1, Figure 4.1, Figure 4.2.

Selected confounders included age at anthropometric measurement (continuous), offspring sex (binary; 0-Male, 1-Female) where appropriate and randomisation to the UPBEAT intervention (Model 1). Further adjustment was made for maternal parity (binary; 0-Nultiparous, 1-Multiparous), ethnicity (categorical; reference white ethnicity), current smoker in early pregnancy (binary; 0-Non/ex-smoker, 1-Current smoker in early pregnancy), GDM (binary; 0-No GDM, 1-GDM), GWG (continuous), within a second model (Model 2). For potential associations of metabolic profile at birth and infant

anthropometry at 6 months of age, further adjustment was made for mode of feeding (categorical; reference exclusive breastfeeding ≥ 4 months of age).

All linear regression models were further assessed for data points exhibiting high leverage by using Cook's Distance (defined as $D_i > 4/n$) (Altman and Krzywinski, 2016), heteroscedasticity and linearity. Correction for multiple testing was undertaken using FDR utilising the Benjamin & Hochberg procedure. Presented significance levels were corrected for multiple testing (statistical significance $p < 0.05$) (Benjamini and Hochberg, 1995).

4.2.6.5 Sensitivity analyses

Sensitivity analysis were undertaken by assessing demographic characteristics for those included within the analyses versus the mother-offspring pairs excluded. It is well documented that preterm neonates have a significantly different metabolic profile at birth, and subsequent growth, therefore a further sensitivity analysis was performed excluding offspring born < 34 weeks' gestation (Tea *et al.*, 2012). A third sensitivity analysis was undertaken, as treatment for GDM has been shown to alter fetal adipokines (Landon *et al.*, 2009); to address this potential effect mothers diagnosed with GDM were excluded from the analysis. As mode of delivery has been shown to influence the cord blood metabolic profile (Godfrey *et al.*, 1996b), a fourth sensitivity analysis was undertaken with statistical models further adjusted for mode of delivery (reference category; unassisted vaginal delivery).

Variables	Reason
Maternal	
UPBEAT Intervention	The intervention was associated with a reduction in maternal GWG, measures of adiposity and improvements in maternal antenatal diet and physical activity (Poston <i>et al.</i> , 2015). In the offspring, the intervention was associated with a reduction in cord insulin and a measure of central adiposity at 6 months of age.
BMI	Increasing maternal BMI is associated with raised cord lipid, inflammation markers and markers associated with insulin resistance as well as a determinants of neonatal body composition (Lemas <i>et al.</i> , 2015; Sharp <i>et al.</i> , 2015).
Parity	Increasing maternal parity is associated with increasing neonatal adiposity as shown in a rural Indian population (Joshi <i>et al.</i> , 2005) and a prospective birth cohort in the Netherlands (Gaillard <i>et al.</i> , 2014a)
Ethnicity	Maternal ethnicity is differentially associated with neonatal adiposity e.g. offspring born to black ethnic groups have reduced birthweight in comparison to those from white ethnic groups (Okosun <i>et al.</i> , 2000; Lin <i>et al.</i> , 2015).
Socioeconomic status	Socioeconomic deprivation is associated with reduced educational attainment and poor maternal lifestyle behaviours, known determinants of neonatal adiposity (Ranjit <i>et al.</i> , 2015).
Smoker in early pregnancy	Smoking in pregnancy is associated with intrauterine growth restriction associated and with an adverse metabolic profile measured in the cord blood (Rolle-Kampczyk <i>et al.</i> , 2016).
GWG	Multiple observational studies have demonstrated a linear association between GWG and neonatal adiposity (Fraser <i>et al.</i> , 2010). A linear relationship with maternal GWG, cord adipokines and markers insulin resistance has been previously demonstrated in offspring born to heterogeneous BMI (Solis-Paredes <i>et al.</i> , 2016).
GDM	The relationship between maternal glycaemia and neonatal adiposity has recently proven causal. Pathway analysis have identified maternal glucose to be key in determining neonatal anthropometry mediated by fetal metabolic profile at birth (Lawlor <i>et al.</i> , 2014).
Neonate	
Sex	Offspring sex has shown to have a differential influence on cord biomarkers of inflammation and adipokines. Females offspring have significantly more adipose tissue in comparison to male offspring (Power and Schulkin, 2008).
Gestation at delivery	Preterm delivery is associated with reduced neonatal birthweight, length and measures of adiposity. The metabolic profile at birth in preterm neonates significantly differs from offspring born at term (Moco <i>et al.</i> , 2013).
Infant	
Mode of early life feeding	Breastfeeding has been demonstrated to be protective against childhood obesity in early life in comparison to offspring formula fed (Owen <i>et al.</i> , 2005).

Table 4.1: A-priori reasoning for selection of maternal, neonatal and infant confounders for the association between cord blood metabolic profile and offspring anthropometry.

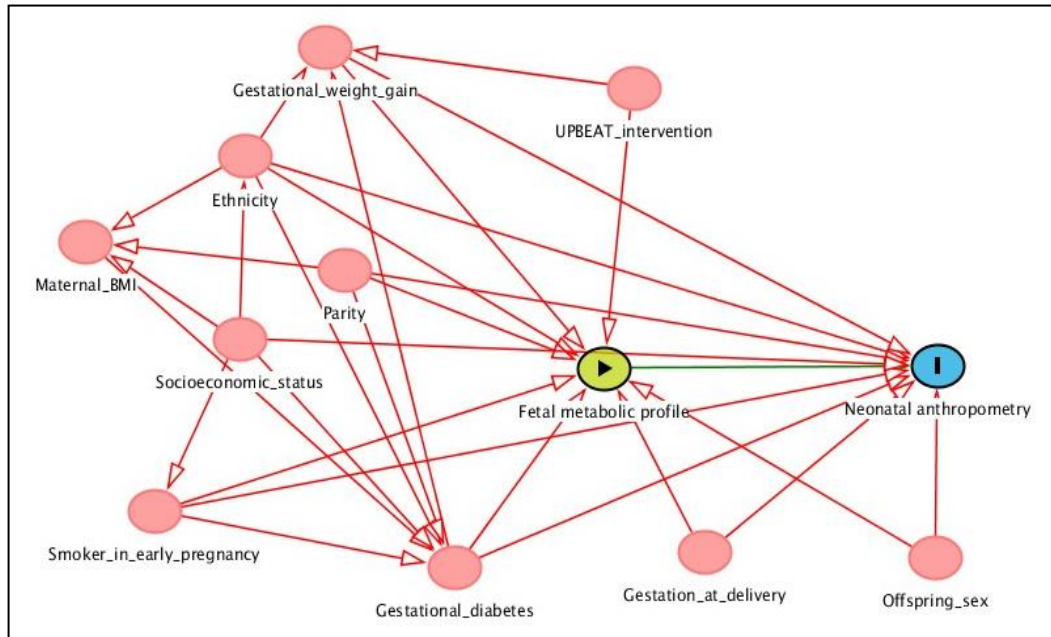


Figure 4.1: Direct acyclic graph to demonstrate the inter-relationships between variables potentially confounding the relationship between cord blood metabolic profile and neonatal anthropometry.

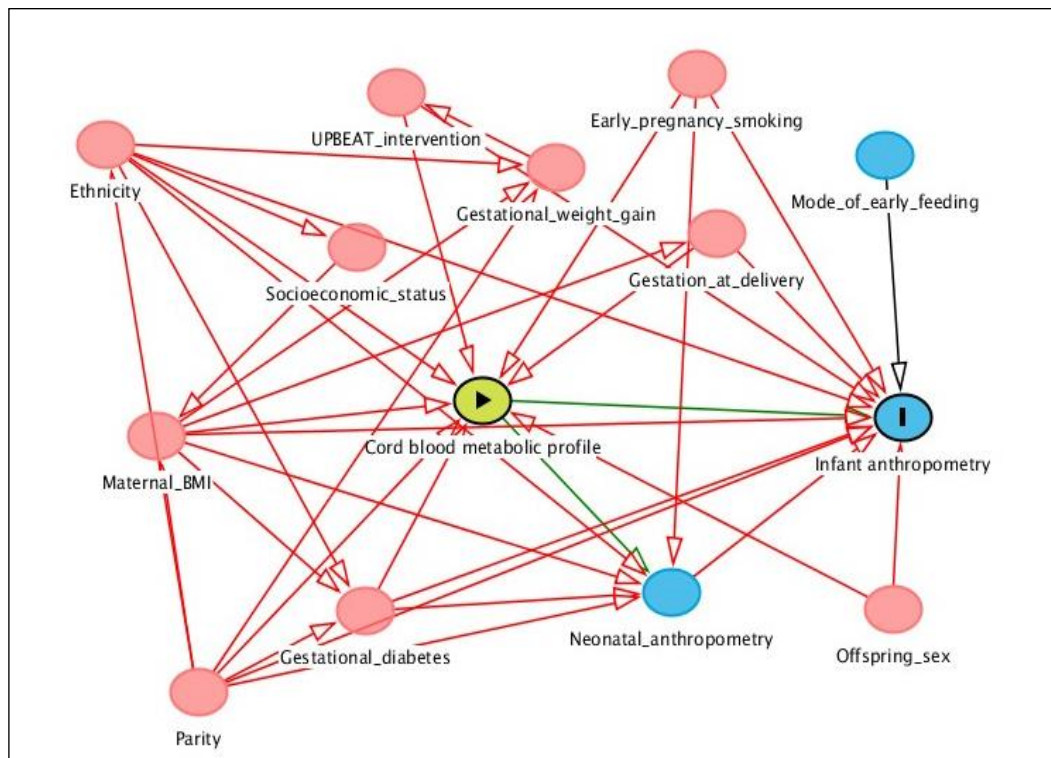


Figure 4.2: Direct acyclic graph to demonstrate inter-relationships between variables potentially confounding the relationship between cord blood metabolic profile and infant anthropometry at 6 months of age.

4.3 Results

4.3.1 Demography

Of the 608 cord samples, available from neonates born to women randomised to the UPBEAT trial; 343 mother-offspring pairs were included within this analyses (Figure 4.3). Median maternal BMI was 35.6 kg/m² (IQR 33.0, 38.9), 71.7% were of a white ethnic group and 87.8% were in the highest quintiles of socioeconomic deprivation. Median neonatal birthweight was 3.5 kg (IQR 3.21, 3.82 kg) and 26.0% of offspring demonstrated significant catch up growth between assessment at birth and 6 month follow-up visit as defined by the WHO (WHO, 2006). Further maternal, neonatal and infant demographics and anthropometric characteristics are provided in Table 4.2. To assess for the potential of selection bias, comparisons were made between mother-offspring pairs included and excluded from the analysis. The incidence of Black ethnicity, neonatal birthweight and subscapular SFT were different between the two groups (Table 4.3). There was no difference in the incidence of GDM, total GWG or infant anthropometric measures between the two groups (Table 4.3).

Summary statistics of cord blood metabolic profile including candidate biomarkers and metabolomic analyses are shown in Table 4.4. Following principal component analysis, 4 distinct clusters of metabolites were identified which were; “Phosphatidylcholines”, “Non-esterified fatty acids”, “Long-chain Acylcarnitines and TCA metabolites” and “Amino acids”, illustrated in Figure 4.4-Figure 4.7.

4.3.2 Effect of the UPBEAT intervention

Mother’s included in this analysis were older, more likely to be nulliparous and less likely to be of Black ethnic origin compared to those without a cord blood sample (Table 4.5). Following correction for multiple testing, there were no significant differences in the cord

blood metabolic profile including clusters derived from principal component analysis, between intervention and control arms (Figure 4.8).

4.3.3 Relationship between maternal antenatal exposures and cord blood metabolic profile

Diagnosis of maternal GDM was associated with reduced cord blood adiponectin and increased isocitric acid and LPC 18.1 concentrations following correction for multiple testing by using a FDR (Figure 4.9). Both maternal early pregnancy BMI and total GWG were not associated with any components of the cord blood metabolic profile (Figure 4.10 & Figure 4.11). Maternal fasting glucose collected at the time of the OGTT was positively associated with higher cord insulin, C-peptide, LPC 18.1, 18.2 and 20.4, alpha aminoadipic acid and citric acid following correction for multiple testing. Maternal fasting glucose was also associated with lower cord adiponectin and NEFA 26.0 (Figure 4.12). Associations between maternal glucose at 1 and 2 hour post OGTT with the cord blood metabolic profile are illustrated in Figure 4.13 and Figure 4.14.

4.3.4 Associations between cord blood metabolic profile and neonatal anthropometry

There was a positive linear relationship between cord C-peptide, insulin, IGF-1, leptin and neonatal birthweight z-scores, SSFT, subscapular SFT, triceps SFT (with the exception of insulin, and C-peptide), mid upper arm and abdominal circumference (Table 4.6, Figure 4.15). Clusters of NEFAs as assessed in the metabolome, and cord blood triglycerides, were inversely associated with neonatal birthweight z-scores, SSFT, subscapular SFT, triceps SFT and mid upper arm circumference at birth (Table 4.6, Figure 4.15). HDL, adiponectin and clusters of phosphatidylcholines were linearly associated with birthweight z-score only. LPC 16.1 and 18.0 were positively associated with neonatal birth weight, SSFT, subscapular and triceps SFTs following correction for multiple testing (Figure 4.16 and Figure 4.17). Cord blood cholesterol was not associated with any measure of neonatal

anthropometry (Figure 4.15). IL-6 and TNF- α were negatively associated with neonatal birthweight z-scores. There were no associations between cord clusters of acylcarnitines, amino acids and IGF-II with any measure of neonatal anthropometry (Table 4.6, Figure 4.15 to Figure 4.17).

4.3.5 Associations between cord blood metabolic profile and infant anthropometry at 6 months of age

Of those cord blood, biochemical variables significantly associated with neonatal body composition, clusters of phosphatidylcholines and adiponectin were linearly associated with infant weight and length z-scores at 6 months of age (Table 4.7). In particular, LPC 16.1 and 18.1 were linearly associated with infant weight z-scores at 6 months of age (Figure 4.16 & Figure 4.17). Cord IGF-I was linearly associated with infant weight, BMI and mid upper arm circumference z-scores (Table 4.7; Figure 4.16, Figure 4.17). Cord leptin and triglycerides were negatively associated with infant mid-upper arm circumference z-scores following adjustment for maternal and infant confounding (Table 4.7; Figure 4.16, Figure 4.17). There were no associations between cord insulin, glucose, C-peptide and IL-6 with infant anthropometry at 6 months of age (Table 4.7; Figure 4.16, Figure 4.17).

For every unit increase in clusters of phosphatidylcholines, the odds of catch up growth at 6 months of age increased by 1.35 (1.04 to 1.75), whereas leptin decreased by 0.33 (0.17 to 0.52) (Table 4.8). IGF-1 and leptin were positively associated with increased odds of catch down growth at 6 months of age (Table 4.8).

4.3.6 *Sensitivity analyses*

The associations between cord blood metabolic profile and neonatal or infant body composition remained unchanged following exclusion of offspring born <34 weeks' gestation (N=36) (Figure 4.18, Figure 4.19), those participants exposed to GDM (N=111) (Figure 4.20, Figure 4.21) and following further adjustment for mode of delivery (Figure 4.22, Figure 4.23).

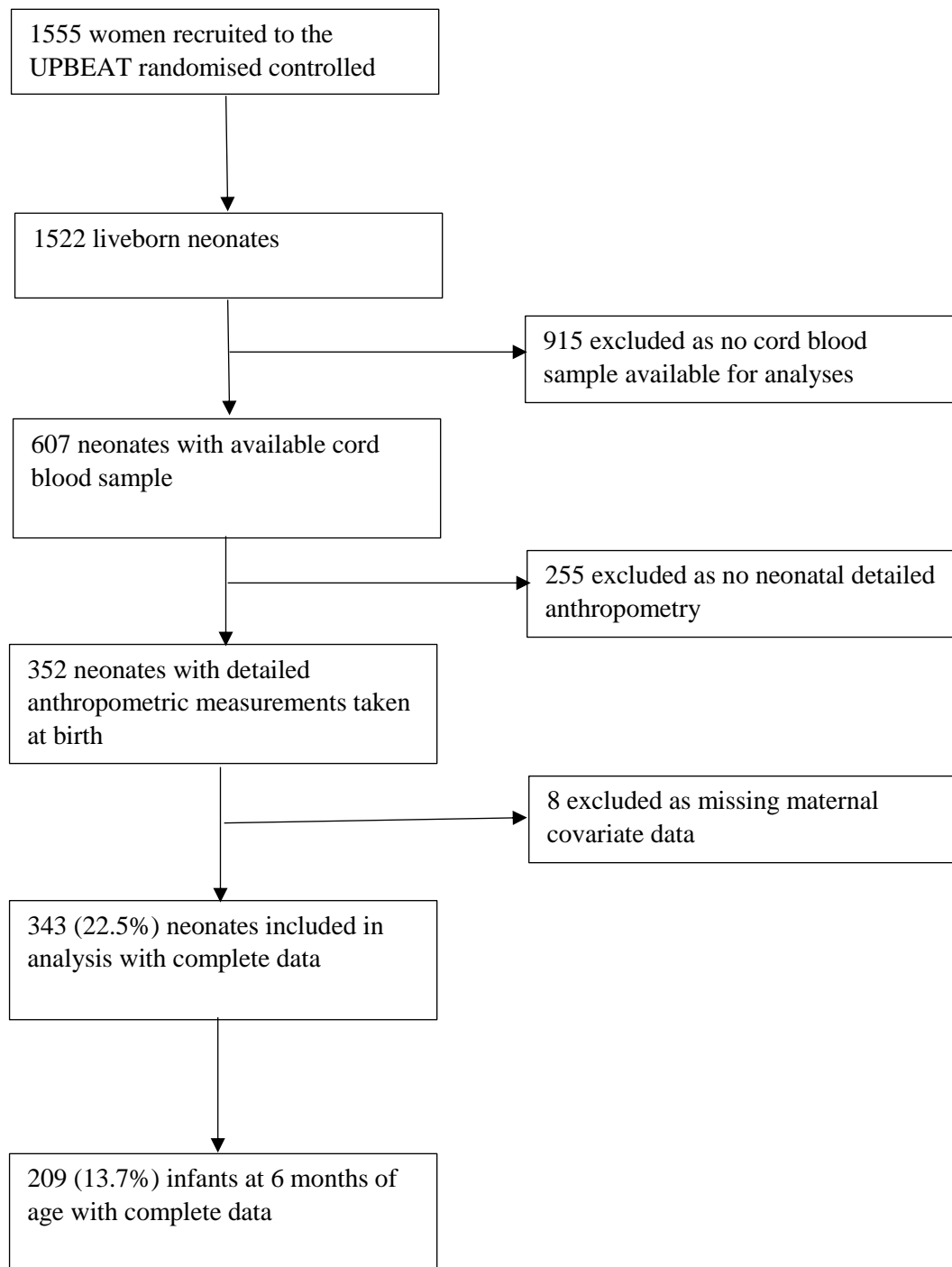


Figure 4.3: Flow diagram of mother-offspring pairs included within this analysis

		N=343	
		N=	Mean (SD)/ Median (IQR)/ N (%)
Maternal			
Age (years)		343	31.0 (27.0, 35.0)
BMI (kg/m ²)		343	35.6 (33.0, 38.9)
Ethnicity	White	343	246 (71.7)
	Black		63 (18.4)
	Asian		19 (5.5)
	Other		15 (4.4)
Multiparous		343	165 (48.1)
Smoker in early pregnancy		343	19 (5.5)
Socioeconomic deprivation		343	295 (87.8)
Gestational weight gain (kg)		343	7.78 (4.07)
Gestational diabetes		343	111 (32.4)
Neonate			
Mode of delivery	Vaginal	343	138 (40.2)
	Operative vaginal		44 (12.8)
	Emergency C-section		78 (22.7)
	Elective C-section		83 (24.2)
Gestation at delivery (weeks)		343	39.9 (38.7, 40.9)
Birthweight (kg)		343	3.6 (3.2, 3.8)
Birthweight z scores		343	0.21 (0.76)
Skinfold thickness-subscapular (mm)		343	5.78 (1.42)
Skinfold thickness triceps (mm)		343	5.34 (1.45)
Sum of skinfold thicknesses (mm)		343	11.12 (2.59)
Midarm circumference (cm)		343	11.58 (0.97)
Abdominal circumference (cm)		343	32.63 (2.05)
Infant			
Weight for age z-score		247	0.29 (1.01)
Length for age z-score		238	0.44 (1.85)
BMI for age z-score		238	0.10 (1.84)
Arm circumference z-score		246	1.25 (1.47)
Triceps skinfold thickness z-score		242	0.29 (1.51)
Subscapular skinfold thickness z-score		209	0.30 (1.45)
Catch up growth*		246	64 (26.0)
Catch down growth**		246	60 (24.4)

Table 4.2: Maternal, neonatal and infant demographic, anthropometry and clinical characteristics of mother-offspring pairs included within this analyses (n=343).

Abbreviations BMI-Body Mass Index. *Catch up growth defined as a ≥ 0.67 SDs increase in weight-z-scores from birth to 6 months of age. **Catch down growth defined as a ≥ 0.67 SDS decrease in weight-z-scores from birth to 6 months of age.

		Included within the analyses (n=343)/ (n=247)	Excluded from analysis (n=1211)	p-value
		Mean (SD)/ Median (IQR)/ N (%)	Mean (SD)/ Median (IQR)/ N (%)	
Maternal				
Age (years)		31.0 (27.0, 35.0)	30.0 (26.0, 35.0)	0.109
BMI (Kg/ m2)		35.6 (33.0, 38.9)	34.9 (32.7, 38.5)	0.150
Ethnicity	Asian	19 (5.5)	76 (6.3)	0.616
	Black	63 (18.4)	338 (27.9)	0.001
	Other	15 (4.4)	70 (5.8)	0.315
	White	246 (71.7)	727 (60.0)	<0.001
Multiparous		165 (48.1)	715 (59.0)	0.001
Smoker in early pregnancy		19 (5.5)	89 (7.3)	0.249
Socioeconomic deprivation		295 (86.5)	1085 (89.9)	0.102
Gestational weight gain (kg)		7.48 (4.07)	7.51 (4.74)	0.891
Gestational diabetes		111 (32.4)	269 (27.3)	0.070
Neonate				
Mode of delivery	Emergency C-section	78 (22.7)	171 (14.5)	0.159
	Operative vaginal	44 (12.8)	134 (11.4)	<0.001
	Elective C-section	83 (24.2)	212 (18.0)	0.010
	Vaginal	138 (40.2)	660 (56.1)	<0.001
Gestation at delivery (weeks)		39.9 (38.7, 40.9)	39.9 (38.7, 40.9)	<0.001
Birthweight (grams)		3555.0 (3210.0, 3827.0)	3435.0 (3090.0, 3780.0)	<0.001
Skinfold thickness subscapular (mm)		5.78 (1.42)	5.39 (1.43)	0.004
Skinfold thickness triceps (mm)		5.34 (1.45)	5.24 (1.61)	0.494
Sum of skinfold thicknesses (mm)		11.12 (2.59)	10.51 (2.70)	0.018
Midarm circumference (cm)		11.58 (0.97)	11.34 (1.24)	0.009
Abdominal circumference (cm)		32.63 (2.05)	32.28 (2.08)	0.061
Infant at 6 months				
Weight for age z-score		0.29 (1.01)	0.24 (1.15)	0.564
Length for age z-score		0.44 (1.85)	0.59 (1.84)	0.317
BMI for age z-score		0.10 (1.84)	-0.09 (1.80)	0.208
Arm circumference z-score		1.25 (1.47)	1.04 (1.76)	0.109
Triceps SFT z-score		0.29 (1.51)	0.09 (1.48)	0.098
Subscapular SFT z-score		0.30 (1.45)	0.17 (1.32)	0.304

Table 4.3: Differences in maternal and neonatal demographic, clinical and anthropometric characteristics between mother-neonatal pairs included and excluded within the analysis.

Abbreviations; BMI- Body Mass Index.

Cord blood biochemical profile	N	Concentrations (Median/ IQR)
<i>Candidate cord blood biomarkers</i>		
C-peptide (ng/ml)	574	1.3 (0.9,1.7)
Insulin (U/ml)	576	6.4 (3.8,10.1)
Glucose (mmol/l)	576	3.4 (2.4,4.2)
Triglycerides (mmol/l)	516	0.3 (0.3,0.5)
Cholesterol (mmol/l)	546	1.4 (1.1,1.7)
HDL (mmol/l)	577	41.0 (29.0,62.0)
IGF I (ng/ml)	577	45.0 (32.0,58.0)
IGF II (ng/ml)	577	496.0 (441.0,558.0)
Leptin (ng/ml)	577	14.5 (8.0,24.0)
Adiponectin (ng/ml)	577	31.5 (23.3,39.5)
IL-6 (pg/ml)	577	4.0 (2.0,8.9)
TNF-alpha (pg/ml)	577	139.0 (87.0,197.0)
<i>Metabolites (μmol/l)</i>		
lyso.PC.a.C14.0	364	1.4 (1.1,1.8)
lyso.PC.a.C16.0	607	50.6 (39.8,61.4)
lyso.PC.a.C16.1	526	4.3 (3.1,5.4)
lyso.PC.a.C18.0	607	9.0 (6.8,11.1)
lyso.PC.a.C18.1	526	10.4 (8.1,13.6)
lyso.PC.a.C18.2	607	11.9 (8.7,15.6)
lyso.PC.a.C18.3	360	0.3 (0.2,0.4)
lyso.PC.a.C20.3	526	3.0 (2.2,4.0)
lyso.PC.a.C20.4	607	13.7 (10.3,18.7)
lyso.PC.a.C20.5	324	0.3 (0.2,0.4)
lyso.PC.a.C22.5	400	0.4 (0.3,0.7)
lyso.PC.a.C22.6	607	2.3 (1.6,3.1)
lyso.PC.e.C18.0	321	0.6 (0.4,0.8)
PC.aa.C30.0	522	2.8 (2.1,3.6)
PC.aa.C30.2	313	0.4 (0.3,0.6)
PC.aa.C32.0	526	16.3 (13.1,21.0)
PC.aa.C32.1	525	13.7 (10.4,18.4)
PC.aa.C32.2	342	0.7 (0.4,1.1)
PC.aa.C34.1	607	126.7 (99.5,161.3)
PC.aa.C34.2	607	70.9 (55.8,87.8)
PC.aa.C34.3	442	3.3 (2.6,4.1)
PC.aa.C34.4	440	0.5 (0.4,0.6)
PC.aa.C36.0	520	1.8 (1.2,2.5)
PC.aa.C36.1	525	22.0 (17.9,27.5)
PC.aa.C36.2	445	42.4 (33.8,52.1)
PC.aa.C36.3	607	57.1 (46.7,71.1)
PC.aa.C36.4	607	123.6 (98.3,152.8)
PC.aa.C36.5	445	3.9 (3.0,5.0)
PC.aa.C38.0	523	1.2 (1.0,1.7)
PC.aa.C38.3	525	35.3 (28.1,42.9)
PC.aa.C38.4	526	96.7 (76.6,119.9)
PC.aa.C38.5	445	20.9 (16.5,26.3)
PC.aa.C38.6	526	58.6 (44.7,75.9)
PC.aa.C40.4	441	3.2 (2.5,4.0)
PC.aa.C40.5	524	6.4 (4.4,8.4)
PC.aa.C40.6	526	27.0 (20.0,36.9)
PC.aa.C42.0	520	0.5 (0.3,0.7)

PC.aa.C43.6	318	0.8 (0.6,1.1)
PC.aa.C44.12	441	0.5 (0.4,0.6)
PC.ae.C32.0	525	2.5 (2.0,3.2)
PC.ae.C32.1	525	2.9 (2.3,3.7)
PC.ae.C32.2	443	0.8 (0.5,1.1)
PC.ae.C34.0	403	1.3 (1.0,1.7)
PC.ae.C34.1	483	4.5 (3.7,5.8)
PC.ae.C34.2	523	2.8 (2.1,3.5)
PC.ae.C34.3	524	1.0 (0.7,1.2)
PC.ae.C36.1	523	2.7 (2.2,3.3)
PC.ae.C36.2	521	2.4 (1.9,2.9)
PC.ae.C36.3	445	1.6 (1.2,2.1)
PC.ae.C36.4	606	7.0 (5.4,8.6)
PC.ae.C36.5	526	5.5 (4.2,7.0)
PC.ae.C38.0	482	1.1 (0.8,1.4)
PC.ae.C38.3	442	2.4 (1.8,3.0)
PC.ae.C38.4	526	7.4 (5.8,9.0)
PC.ae.C38.5	445	6.0 (4.8,7.5)
PC.ae.C38.6	443	2.8 (2.1,3.5)
PC.ae.C40.0	484	6.4 (4.9,7.9)
PC.ae.C40.1	403	1.0 (0.5,1.4)
PC.ae.C40.4	521	1.7 (1.3,2.2)
PC.ae.C40.5	604	1.7 (1.3,2.3)
PC.ae.C40.6	524	2.2 (1.7,2.8)
PC.ae.C42.2	322	0.3 (0.2,0.4)
PC.ae.C42.5	402	1.0 (0.8,1.4)
PC.ae.C42.6	440	0.6 (0.4,0.9)
SM.a.C32.1	525	2.7 (2.1,3.5)
SM.a.C33.1	439	1.8 (1.4,2.3)
SM.a.C34.0	443	3.4 (2.7,4.2)
SM.a.C34.1	607	60.8 (47.2,80.5)
SM.a.C34.2	525	9.7 (7.6,12.3)
SM.a.C35.1	364	1.5 (1.2,2.0)
SM.a.C36.1	526	18.3 (14.3,22.4)
SM.a.C36.2	606	12.1 (8.9,15.4)
SM.a.C38.1	324	15.6 (11.9,31.6)
SM.a.C38.2	523	14.2 (11.7,17.6)
SM.a.C39.1	477	1.3 (1.0,1.7)
SM.a.C40.2	483	18.5 (14.0,26.5)
SM.a.C40.4	520	2.0 (1.4,2.9)
SM.a.C41.1	364	3.9 (3.1,4.8)
SM.a.C41.2	445	2.7 (2.1,3.4)
SM.a.C42.1	425	23.9 (19.3,28.7)
SM.a.C42.2	605	31.7 (25.8,38.7)
SM.a.C42.3	605	19.1 (15.5,24.4)
SM.a.C42.6	359	3.6 (2.6,4.7)
SM.a.C43.1	520	1.1 (0.8,1.4)
SM.a.C44.1	305	0.6 (0.1,1.8)
SM.a.C41.0	401	0.7 (0.4,1.7)
SM.a.C47.5	481	0.0 (0.0,0.0)
SM.a.C47.6	314	0.0 (0.0,0.0)
NEFA 10.0	526	0.9 (0.5,1.3)
NEFA 12.0	605	1.9 (1.2,2.8)
NEFA 14.1	524	1.1 (0.5,1.7)

Table 4.4 continued

NEFA 15.1	371	0.0 (0.0,0.0)
NEFA 16.0	607	61.8 (42.9,85.4)
NEFA 16.1	607	4.2 (2.7,6.9)
NEFA 17.0	607	2.0 (1.5,2.5)
NEFA 17.1	526	0.5 (0.4,0.8)
NEFA 18.1	607	51.3 (35.2,73.4)
NEFA 18.2	524	16.4 (10.8,25.8)
NEFA 18.3	583	1.1 (0.7,1.9)
NEFA 20.0	481	0.4 (0.3,0.5)
NEFA 20.1	604	0.6 (0.4,0.9)
NEFA 20.2	526	0.7 (0.5,0.9)
NEFA 20.3	607	1.5 (1.1,2.2)
NEFA 20.4	606	2.3 (1.6,3.6)
NEFA 22.0	526	0.2 (0.1,0.2)
NEFA 22.1	597	0.1 (0.1,0.2)
NEFA 22.2	596	0.1 (0.1,0.1)
NEFA 22.6	521	3.2 (2.2,4.6)
NEFA 24.1	598	0.4 (0.3,0.6)
NEFA 19.0	441	0.4 (0.3,0.5)
NEFA 19.1	523	0.3 (0.2,0.4)
NEFA 20.5	451	0.1 (0.0,0.2)
NEFA 22.3	402	0.3 (0.2,0.4)
NEFA 22.4	599	0.7 (0.5,1.0)
NEFA 22.5	599	0.6 (0.4,0.9)
NEFA 24.0	517	0.2 (0.1,0.2)
NEFA 24.2	323	0.1 (0.1,0.2)
NEFA 24.4	601	0.2 (0.2,0.3)
NEFA 24.5	524	0.2 (0.1,0.2)
NEFA 24.6	402	0.0 (0.0,0.1)
NEFA 26.0	356	0.0 (0.0,0.1)
NEFA 26.1	479	0.1 (0.1,0.1)
NEFA 26.2	517	0.1 (0.1,0.1)
NEFA 26.3	440	0.1 (0.1,0.1)
NEFA 26.4	519	0.2 (0.1,0.2)
NEFA 26.6	359	0.1 (0.1,0.1)
Carn	525	17.3 (14.3,21.6)
Carn.a.C10.0	525	0.1 (0.1,0.1)
Carn.a.C10.1	525	0.1 (0.1,0.2)
Carn.a.C12.0	524	0.1 (0.0,0.1)
Carn.a.C12.1	442	0.1 (0.1,0.2)
Carn.a.C14.0	606	0.1 (0.0,0.1)
Carn.a.C14.1	525	0.1 (0.1,0.1)
Carn.a.C15.0	401	0.0 (0.0,0.0)
Carn.a.C16.0	602	0.1 (0.1,0.2)
Carn.a.C16.1	605	0.1 (0.1,0.1)
Carn.a.C18.0	442	0.0 (0.0,0.0)
Carn.a.C18.1	604	0.1 (0.1,0.2)
Carn.a.C18.2	357	0.1 (0.1,0.3)
Carn.a.C2.0	525	5.1 (3.9,7.0)
Carn.a.C20.0	397	0.0 (0.0,0.0)
Carn.a.C20.4	304	0.0 (0.0,0.0)
Carn.a.C3.0	605	0.4 (0.3,0.6)
Carn.a.C3.0.DC	444	0.3 (0.2,0.9)
Carn.a.C4.0	605	0.2 (0.1,0.2)

Table 4.4 continued

Carn.a.C4.0.DC	442	0.0 (0.0,0.0)
Carn.a.C5.0	606	0.1 (0.1,0.2)
Carn.a.C6.0	606	0.1 (0.1,0.1)
Carn.a.C8.0	444	0.1 (0.1,0.1)
Carn.a.C8.1	526	0.1 (0.1,0.1)
Carn.a.C9.0	441	0.0 (0.0,0.0)
Pyruvic.acid	605	15.5 (11.2,22.5)
Lactic.acid	605	27.7 (20.1,41.2)
Fumaric.acid	605	47.3 (36.2,64.4)
X3.Methyl.2.oxobutanoic.acid	606	240.5 (115.0,466.0)
Succinic.acid	606	5600.0 (3210.0,10200.0)
Methyl.Malonic.acid	605	1.6 (1.2,2.3)
Taurine	605	6.5 (4.9,9.5)
X3.Methyl.2.oxovaleric.acid	603	0.3 (0.3,0.4)
X4.Methyl.2.oxovaleric.acid	605	121.0 (86.8,173.0)
Malic.acid	604	7.1 (5.4,10.7)
Alpha Ketoglutaric acid	605	4.3 (3.0,6.2)
Alpha Amino adipic acid	604	0.7 (0.5,0.9)
Isocitric acid	604	21.2 (17.4,25.5)
Citric acid	604	114.0 (96.5,133.0)
Beta Hydroxybutyric acid	524	138.0 (63.5,240.0)
Alanine	605	454.0 (367.0,555.0)
Arginine	606	36.0 (21.5,52.1)
Asparagine	605	41.5 (33.2,52.6)
Aspartic acid	605	13.8 (9.0,22.1)
Citrulline	606	13.8 (10.7,17.3)
Glutamine	605	400.0 (322.0,497.0)
Glutamic acid	601	106.0 (78.1,149.0)
Glycine	604	276.5 (231.0,327.0)
Histidine	603	154.0 (125.0,188.0)
Isoleucine	602	61.3 (50.0,77.1)
Leucine	606	107.5 (92.3,128.0)
Lysine	606	230.0 (193.0,288.0)
Methionine	606	28.3 (23.4,35.4)
Ornithine	511	108.0 (85.0,132.0)
Phenylalanine	606	82.8 (70.3,96.7)
Proline	485	186.0 (156.0,243.0)
Tryptophan	605	49.2 (40.3,59.9)
Serine	484	98.0 (79.5,123.0)
Threonine	562	202.5 (162.0,253.0)
Tyrosine	606	58.8 (49.3,73.2)
Valine	606	204.0 (176.0,250.0)
Cysteine	483	14.0 (11.4,17.2)

Table 4.4: Summary statistics of the cord blood metabolic profile including candidate biomarkers and metabolome from obese pregnant women included in the UPBEAT trial (n=607)

Abbreviations; CARN-Carnitines; HDL-High density lipoprotein; IGF I- Insulin growth factor I; IGF II- Insulin growth factor II; IL6- Interleukin 6; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines; SM-sphingomyelins; TNFalpha- Tumor necrosis factor; X30B- x3methyl2oxobutanoicacid; X30V- 3methyl2oxovalericacid; X40B- 4methyl2oxovalericacid.

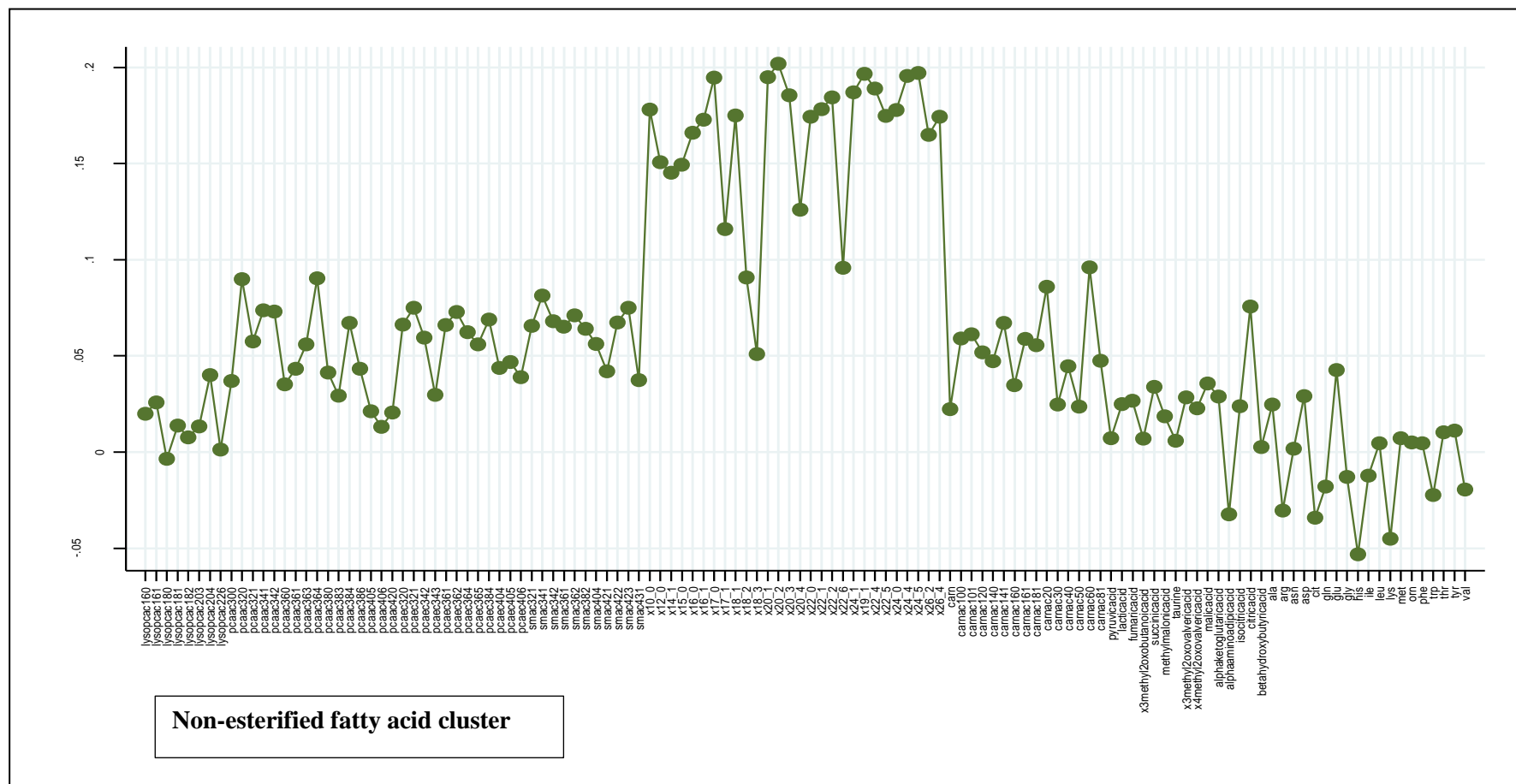


Figure 4.5: Graphical representation of non-esterified fatty acid cluster derived from principal component analysis of the cord blood metabolomic profile from obese pregnant women (n=607).

	Included N=607	Excluded N=947	Comparison (95% CI)	p-value
Maternal age	30.97 (5.39)	30.17 (5.58)	0.81 (0.25 to 1.36)	0.005
Multiparity	318 (52.4%)	562 (59.3%)	0.75 (0.61 to 0.93)	0.007
BMI (kg/m ²)	36.52 (4.93)	36.15 (4.66)	0.37 (-0.12 to 0.86)	0.138
Socioeconomic deprivation	341 (80.0%)	N=822; 666 (80.6%)	0.96 (0.72 to 1.29)	0.806
White ethnicity	433 (71.6)	524 (57.3)	1.25 (1.16 to 1.35)	<0.001
Black ethnicity	116 (19.1%)	272 (29.8%)	0.56 (0.43 to 0.71)	<0.001
Asian ethnicity	29 (4.8%)	62/ (6.8%)	0.69 (0.44 to 1.08)	0.107
Another ethnicity	28 (4.6%)	56 (6.1%)	0.74 (0.46 to 1.18)	0.205
Smoker in early pregnancy	33 (6.4)	66(7.2)	0.88 (0.58 to 1.33)	0.545

Table 4.5: Baseline differences in maternal demographic characteristics at trial entry of those included versus those excluded in the analyses to assess the effect of the UPBEAT intervention on the cord blood metabolic profile.

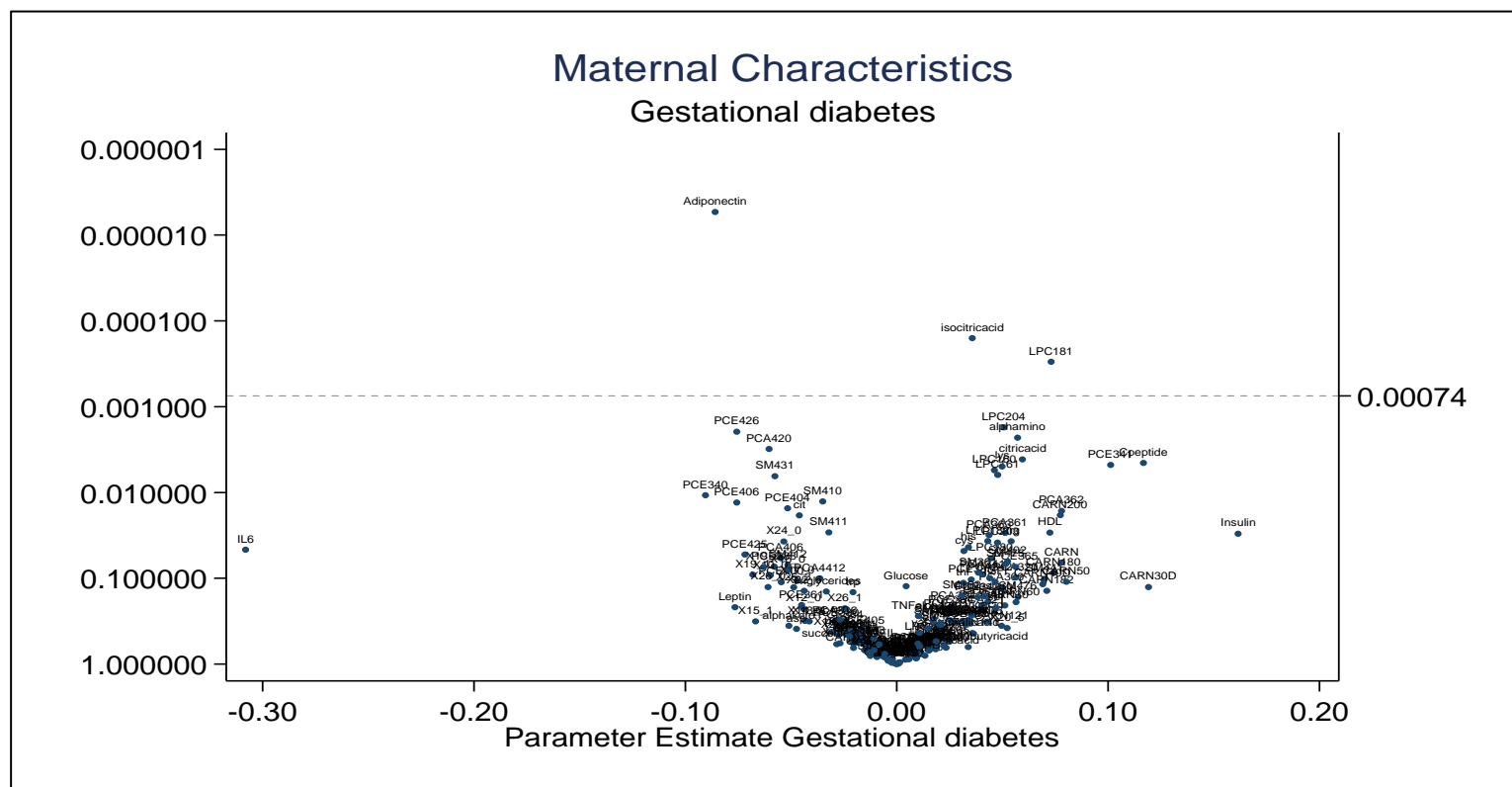


Figure 4.9: Volcano plot demonstrating the association of maternal gestational diabetes with cord blood metabolic profile in obese pregnant women (n=343).

Parameter estimates are graphically represented for each biochemical variable in relation to maternal clinical characteristics following correction for a false discovery rate (Benjamin & Hochberg procedure) (Benjamini and Hochberg, 1995). Adjustment made for randomisation to the UPBEAT intervention, offspring sex and gestation at delivery. Statistical significance $p \leq 0.00074$. Abbreviations; CARN-Carnitines; HDL-High density lipoprotein; IGF1- Insulin growth factor I; IGF II- Insulin growth factor II; IL6- Interleukin 6; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines; SM- sphingomyelins. TNFalpha- Tumor necrosis factor; X30B- x3methyl2oxobutanoicacid; X30V-- x4methyl2oxovalericacid. NEFAs are described using the nomenclature CX: Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.

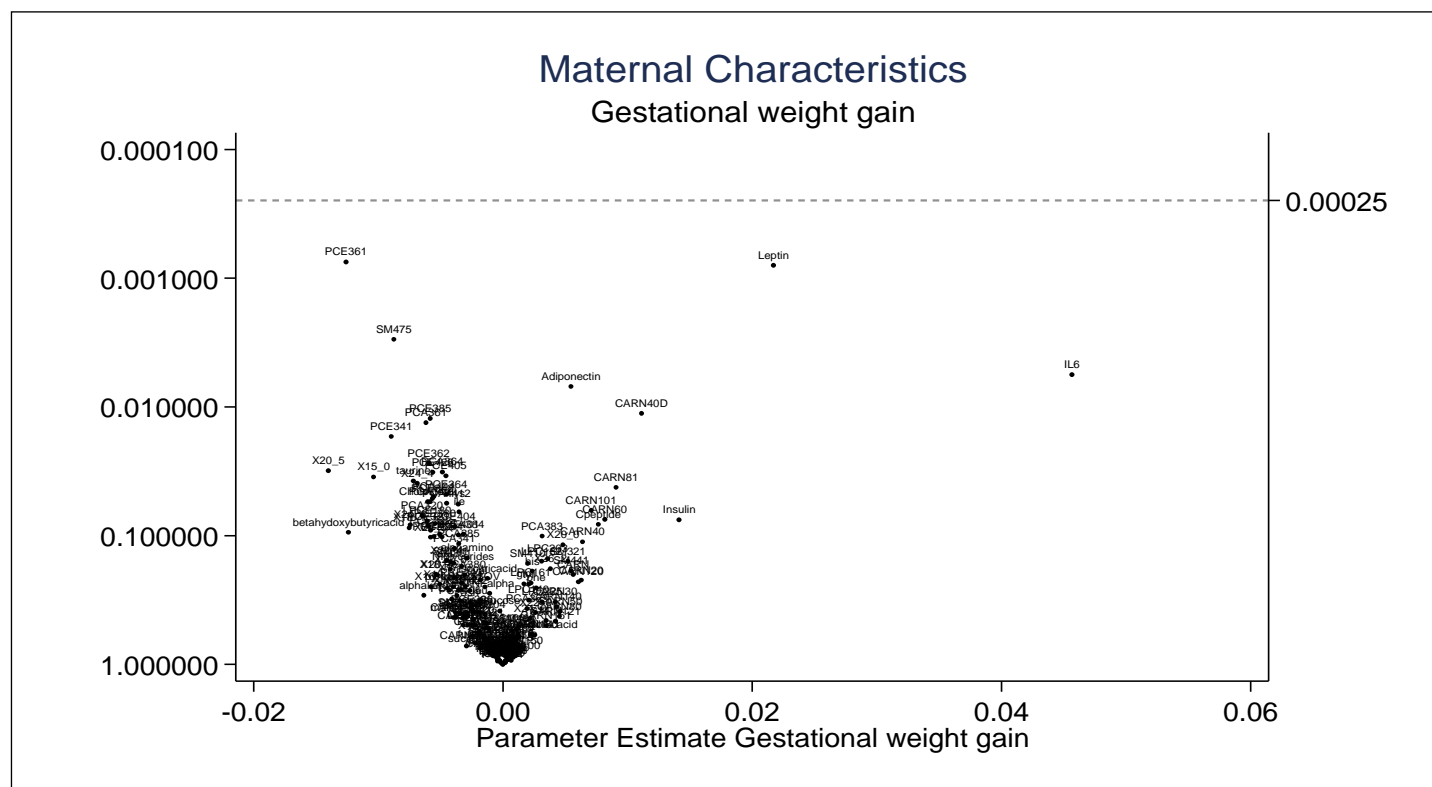


Figure 4.11: Volcano plot demonstrating the association of maternal gestational weight gain with cord blood metabolic profile in obese pregnant women (n=343).

Parameter estimates are graphically represented for each biochemical variable in relation to maternal clinical characteristics following correction for a false discovery rate (Benjamin & Hochberg procedure) (Benjamini and Hochberg, 1995). Adjustment made for randomisation to the UPBEAT intervention, offspring sex and gestation at delivery Statistical significance $p \leq 0.00025$. Abbreviations; CARN-Carnitines; HDL-High density lipoprotein; IGFI- Insulin growth factor I; IGF II- Insulin growth factor II; IL6- Interleukin 6; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines; SM- sphingomyelins. TNFalpha- Tumor necrosis factor; X30B- x3methyl2oxobutanoicacid; X30V-- x4methyl2oxovalericacid. NEFAs are described using the nomenclature CX:Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.

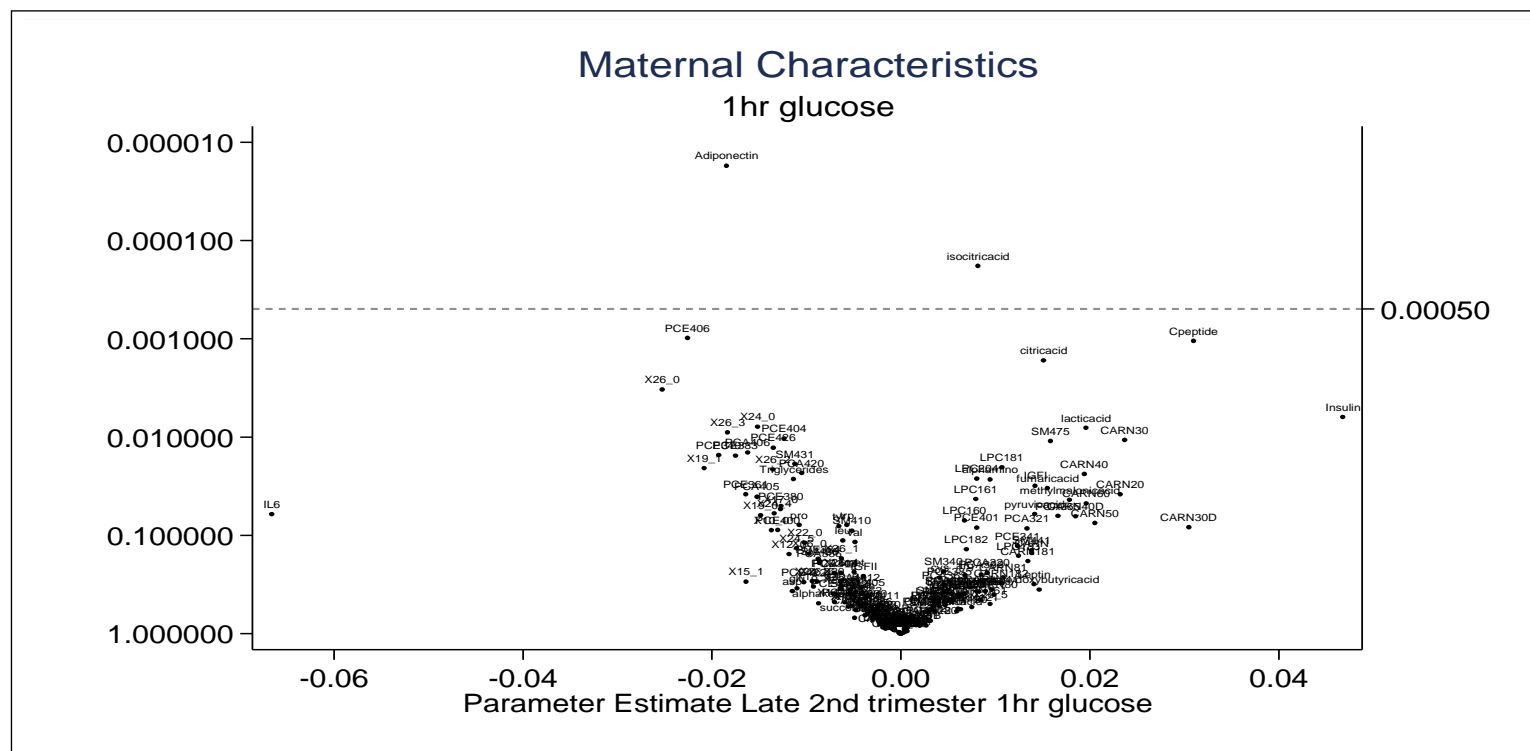


Figure 4.13: Volcano plot demonstrating the association of maternal glucose at 1 hour at the time of the oral glucose tolerance test with cord blood metabolic profile in obese pregnant women (n=343).

Parameter estimates are graphically represented for each biochemical variable in relation to maternal clinical characteristics following correction for a false discovery rate (Benjamin & Hochberg procedure) (Benjamini and Hochberg, 1995). Adjustment made for randomisation to the UPBEAT intervention, offspring sex and gestation at delivery. Statistical significance $p \leq 0.005$. Abbreviations; CARN-Carnitines; HDL-High density lipoprotein; IGFI- Insulin growth factor I; IGF II- Insulin growth factor II; IL6- Interleukin 6; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines; SM- sphingomyelins. TNFalpha- Tumor necrosis factor; X30B- x3methyl2oxobutanoicacid; X30V-- x4methyl2oxovalericacid. NEFAs are described using the nomenclature CX:Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.

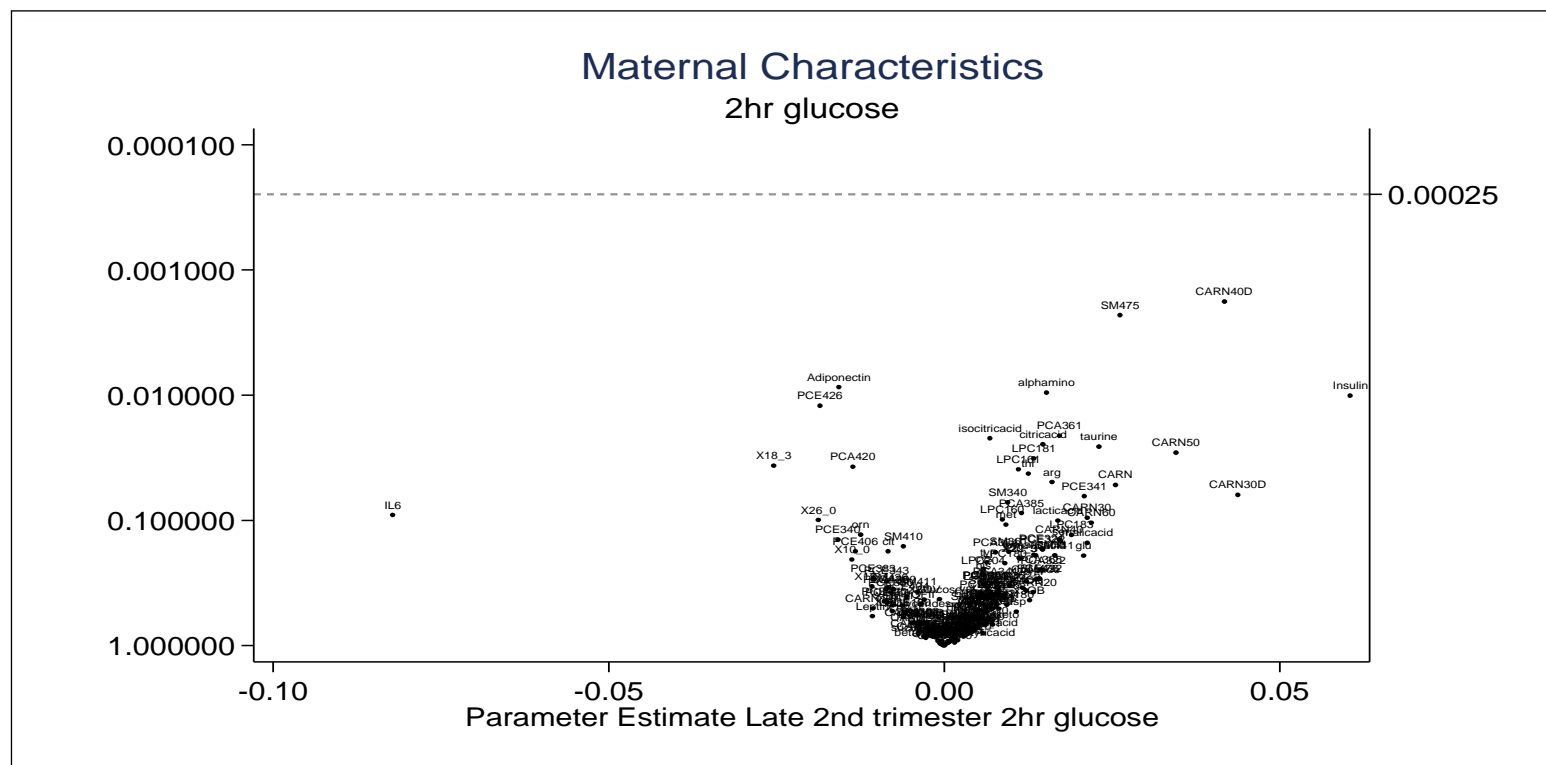


Figure 4.14: Volcano plot demonstrating the association of maternal glucose at 2 hours at the time of the oral glucose tolerance test with cord blood metabolic profile in obese pregnant women (n=343).

	Birthweight z-scores (SDS)	SSFT (mm)	Subscapular SFT (mm)	Triceps SFT (mm)	MUAC (cm)	Abdominal circumference (cm)
	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)
PCA- Phosphatidylcholines	0.04 (0.02 to 0.07) **	-0.11 (-0.25 to 0.03)	-0.06 (-0.12 to 0.01)	-0.05 (-0.15 to 0.04)	0.03 (-0.02 to 0.07)	0.01 (-0.10 to 0.11)
PCA- NEFA	-0.04 (-0.08 to 0.00) *	-0.28 (-0.49 to -0.08) *	-0.11 (-0.21 to 0.00) *	-0.18 (-0.32 to -0.03) *	0.02 (-0.05 to 0.10)	-0.01 (-0.17 to 0.15)
PCA- Long chain acylcarnitine's and TCA metabolites	0.00 (-0.04 to 0.04)	-0.05 (-0.27 to 0.17)	-0.02 (-0.13 to 0.09)	-0.03 (-0.18 to 0.12)	-0.01 (-0.08 to 0.07)	0.01 (-0.15 to 0.17)
PCA-Amino acids	0.05 (0.00 to 0.11)	-0.05 (-0.36 to 0.25)	0.06 (-0.09 to 0.20)	-0.11 (-0.32 to 0.10)	0.06 (-0.04 to 0.16)	0.19 (-0.03 to 0.40)
Peptide (log ₂) ng/ml	0.27 (0.14 to 0.39) **	0.86 (0.22 to 1.50) *	0.52 (0.17 to 0.87) *	0.34 (-0.02 to 0.69)	0.25 (0.00 to 0.49) *	0.73 (0.22 to 1.25) *
Insulin (log ₂) U/ml	0.16 (0.09 to 0.23) **	0.47 (0.12 to 0.82) *	0.31 (0.11 to 0.50) *	0.17 (-0.03 to 0.37)	0.16 (0.02 to 0.29) *	0.38 (0.10 to 0.67) *
Glucose (log ₂) mmol/l	-1.80 (-3.59 to 0.00) *	-2.94 (-12.96 to 7.08)	-3.68 (-9.32 to 1.96)	0.48 (-4.94 to 5.90)	-2.13 (-6.01 to 1.75)	-6.19 (-14.48 to 2.09)
Triglycerides mmol/l	-0.47 (-0.74 to -0.20) **	-1.65 (-3.18 to -0.11) *	-0.95 (-1.82 to -0.08)*	-0.73 (-1.56 to 0.10)	-0.90 (-1.48 to -0.33) *	-1.05 (-2.32 to 0.22)
Cholesterol mmol/l	0.13 (-0.06 to 0.32)	-0.75 (-1.68 to 0.17)	-0.45 (-0.97 to 0.07)	-0.31 (-0.81 to 0.19)	-0.11 (-0.48 to 0.25)	0.40 (-0.38 to 1.17)
HDL mmol/l	0.22 (0.06 to 0.38) *	-0.11 (-0.89 to 0.67)	-0.25 (-0.68 to 0.17)	0.16 (-0.28 to 0.60)	0.02 (-0.27 to 0.32)	0.38 (-0.25 to 1.01)
IGFI (log ₂) ng/ml	1.13 (0.97 to 1.29) **	1.80 (0.89 to 2.72) **	1.08 (0.58 to 1.59) **	0.72 (0.20 to 1.24) *	1.19 (0.87 to 1.51) **	2.41 (1.70 to 3.12) **
IGFII (log ₂) ng/ml	0.31 (-0.02 to 0.65)	1.57 (-0.15 to 3.29)	0.68 (-0.27 to 1.63)	0.89 (-0.08 to 1.85)	0.29 (-0.37 to 0.96)	0.54 (-0.86 to 1.93)
Leptin (log ₂) ng/ml	0.38 (0.30 to 0.46) **	1.59 (1.21 to 1.97) **	0.83 (0.62 to 1.04) **	0.76 (0.54 to 0.98)**	0.50 (0.35 to 0.66) **	0.80 (0.47 to 1.13) **
Adiponectin (log ₂) up/ml	0.40 (0.12 to 0.68)	0.64 (-0.79 to 2.08)	0.48 (-0.31 to 1.27)	0.07 (-0.73 to 0.87)	0.84 (0.31 to 1.37) *	1.16 (0.00 to 2.31)*
IL6 (log ₂) pg/ml	-0.04 (-0.07 to 0.00)*	0.06 (-0.11 to 0.23)	-0.02 (-0.11 to 0.08)	0.08 (-0.02 to 0.17)	-0.04 (-0.11 to 0.03)	-0.14 (-0.28 to 0.00)*
TNF alpha (log ₂) pg/ml	-0.58 (-1.12 to -0.03)*	-1.44 (-4.21 to 1.32)	-0.72 (-2.25 to 0.80)	-0.86 (-2.41 to 0.69)	-0.84 (-1.87 to 0.19)	-0.61 (-2.82 to 1.60)

Table 4.6: Associations of the cord blood metabolic profile with neonatal anthropometry in obese pregnant women (n=343)

Regression coefficients with corresponding 95% confidence intervals presented are adjusted for maternal parity, ethnicity, smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention. Abbreviations; AA-Amino acids; AcylCar-Acylcarnitines; HDL- High density lipoprotein; IGFI- Insulin growth factor I; IGFII- Insulin growth factor II; IL6-Interleukin 6; MUAC-Mid upper arm circumference; NEFA- Non-esterified fatty acids; PC-phosphatidylcholines; PCA-Principal component analysis; SDS- Standard deviation scores; SFT- skinfold thickness; TCA- Tricarboxylic acid; TNFalpha- Tumour Necrosis Factor alpha. * $p < 0.05$; ** $p < 0.001$.

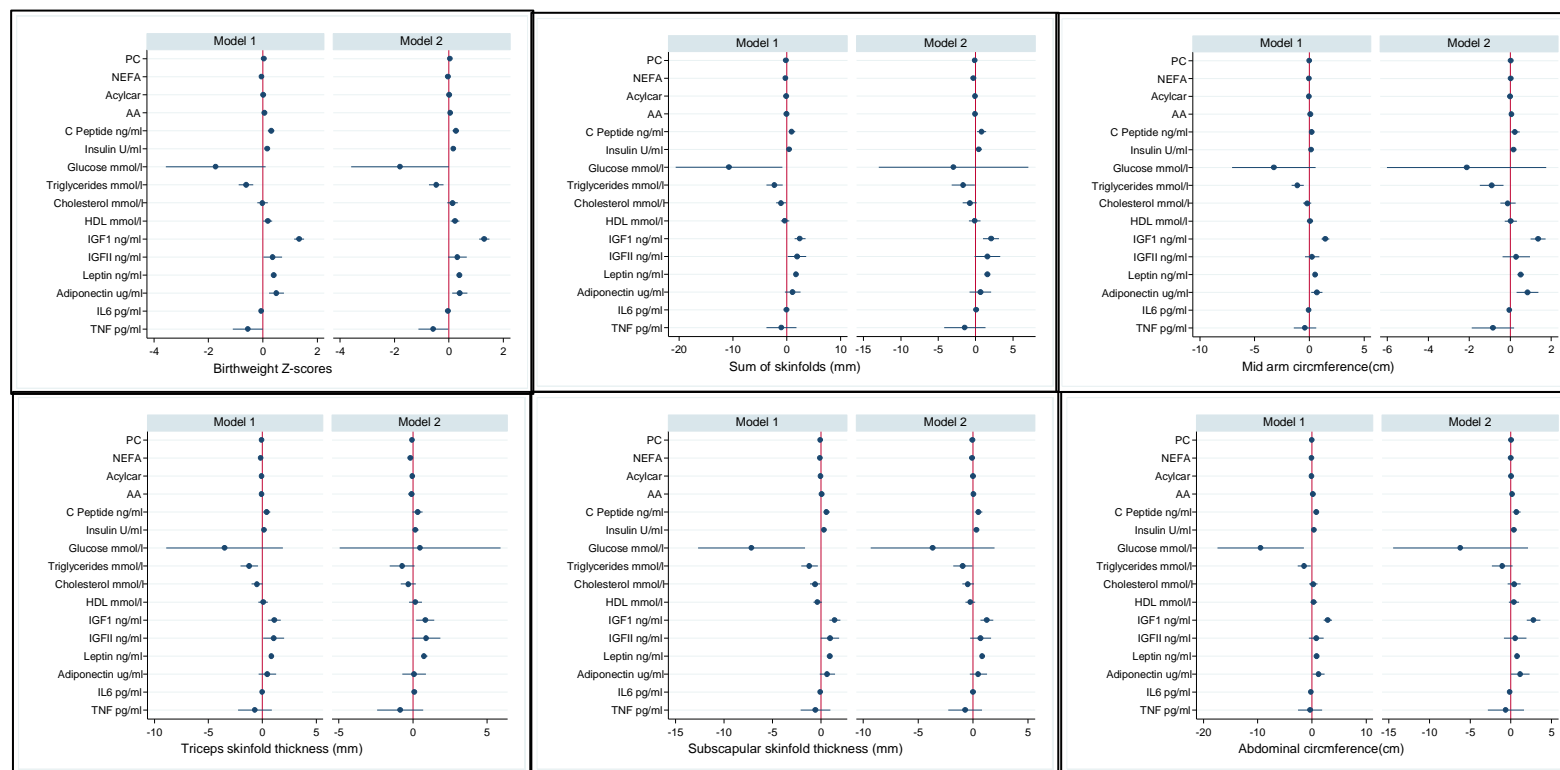


Figure 4.15: Associations of the cord blood metabolic profile with neonatal anthropometry in obese pregnant women (n=343)

Regression coefficients graphically presented with corresponding 95% confidence intervals. Model 1: Adjustment made for offspring sex, gestation at delivery and randomisation to the UPBEAT intervention. Model 2: Adjustment made for maternal parity, ethnicity, and smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention. Abbreviations; AA-Amino acids cluster; AcylCar-Acylcarnitines cluster; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IGFII- Insulin growth factor II; IL6-Interleukin 6; MUAC-Mid upper arm circumference; NEFA- Non-esterified fatty acids cluster; PC-phosphatidylcholines cluster; SDS- Standard deviation scores; SFT- skinfold thickness; TCA- Tricarboxylic acid; TNFalpha- Tumour Necrosis Factor alpha.

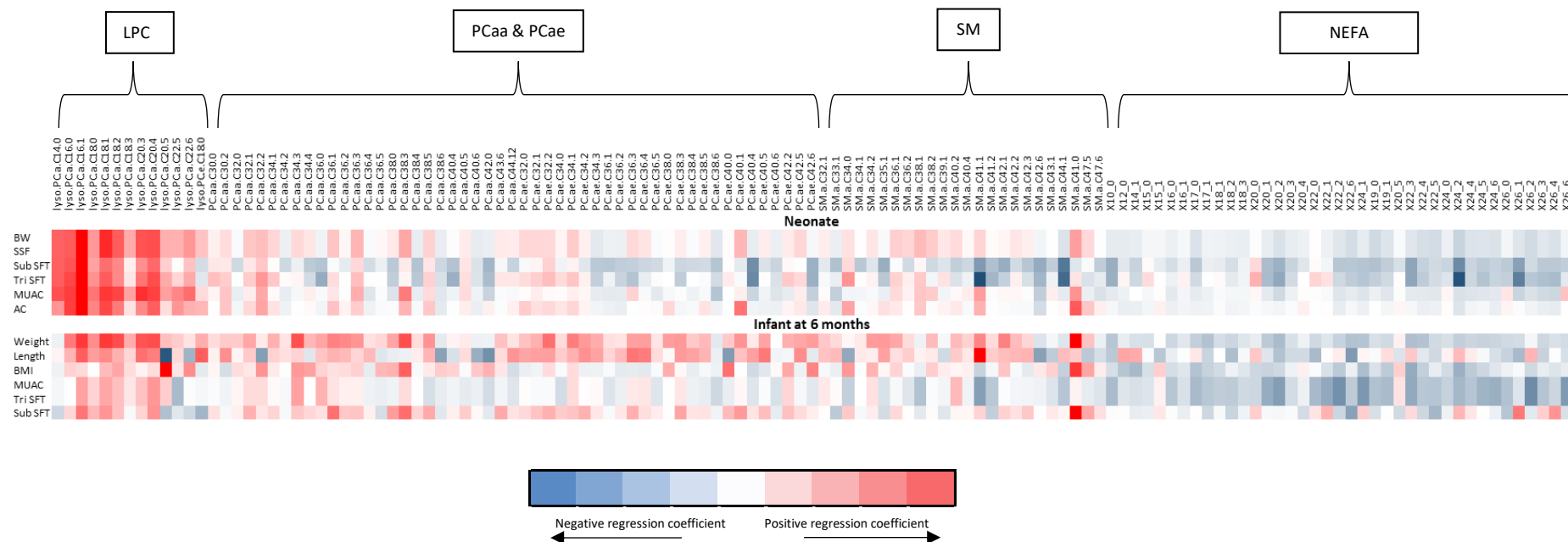


Figure 4.16: Heat map demonstrating associations between cord blood metabolites with anthropometric measurements in neonates (n=343) and infants (n=209); data from obese pregnant women in the UPBEAT study.

Regression coefficients plots with adjustment made for maternal parity, ethnicity, smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention. Additional adjustment is made for early mode of infant feeding for infant anthropometry data at 6 months of age. Abbreviations; AC- Abdominal circumference; BMI-Body mass index; BW- Neonatal birthweight z-scores (SDs); CARN-Carnitines; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; MUAC- Mid upper arm circumference (cm); PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines SM- sphingomyelins; SSF- Sum of skinfold thicknesses (mm); Tri SFT- Triceps skinfold thickness (mm); Sub SFT- Subscapular skinfold thickness (mm); X30B- 3methyl2oxobutanoicacid; X30V- 3methyl2oxovalericacid; X40B- x4methyl2oxovalericacid. NEFAs are described using the nomenclature CX:Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.

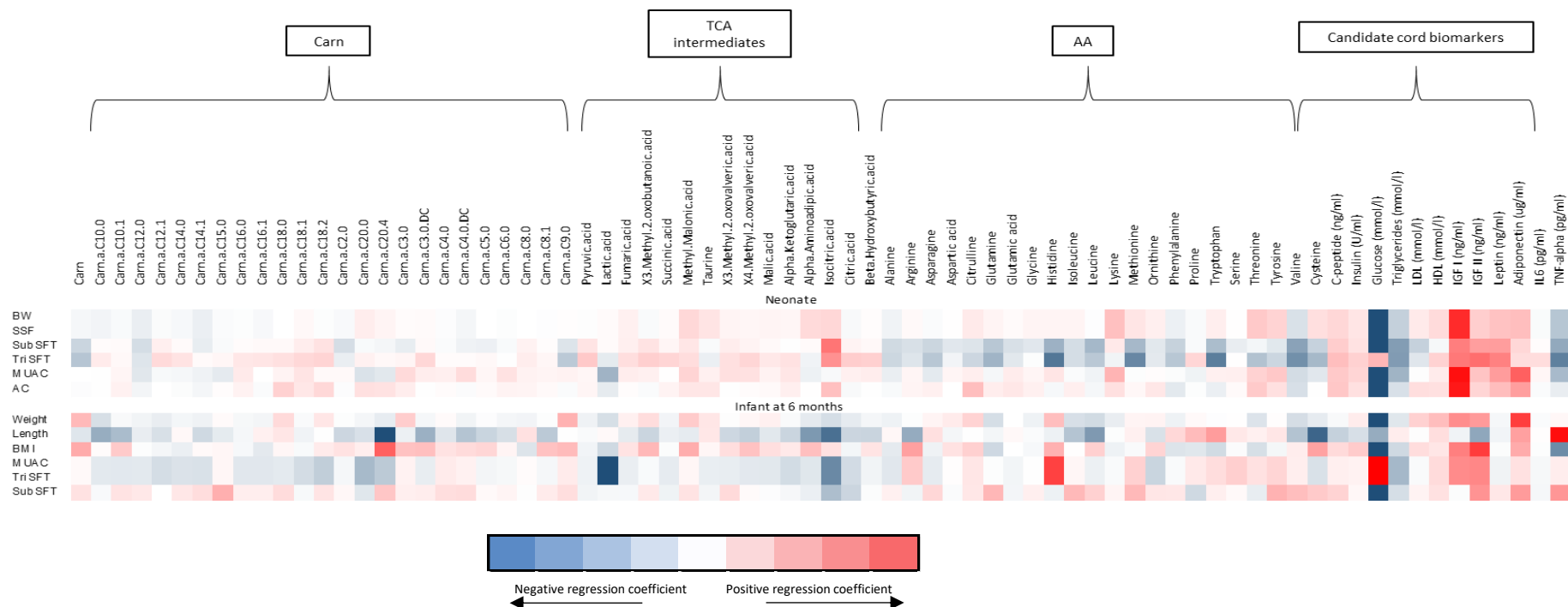


Figure 4.17: Heat map demonstrating associations between cord blood metabolites with anthropometric measurements in neonates (n=343) and infants (n=209); data from obese pregnant women in the UPBEAT study.

Regression coefficients plots with adjustment made for maternal parity, ethnicity, smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention. Additional adjustment is made for early mode of infant feeding for infant anthropometry data at 6 months of age. Abbreviations; AC- abdominal circumference BW- Neonatal birthweight z-scores (SDs); CARN-Carnitines; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; MUAC- Mid upper arm circumference (cm); PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines SM- sphingomyelins; SSF- Sum of skinfold thicknesses (mm); TCA- tricarboxylic acid; Tri SFT- Triceps skinfold thickness (mm); Sub SFT- Subscapular skinfold thickness (mm); X30B- 3methyl2oxobutanoic acid; X30V- 3methyl2oxovaleric acid; X40B- 4methyl2oxovaleric acid. NEFAs are described using the nomenclature CX:Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.

	Weight z-scores (n=247)	Length z-score (n=238)	BMI z-score (n=238)	MUAC z-score (n=246)	Triceps SFT z- score (n=242)	Subscapular SFT z- score (n=209)
	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)	Coef (95% CI)
PCA-Phosphatidylcholines	0.05 (0.00 to 0.10)*	0.07 (0.01 to 0.13)*	0.01 (-0.04 to 0.07)	0.04 (0.00 to 0.08)	0.05 (-0.02 to 0.13)	-0.03 (-0.11 to 0.05)
PCA-NEFA	-0.03 (-0.09 to 0.04)	-0.02 (-0.10 to 0.06)	-0.02 (-0.09 to 0.05)	-0.05 (-0.10 to 0.01)	-0.04 (-0.15 to 0.08)	0.01 (-0.11 to 0.14)
Cpeptide (log ₂) ng/ml	0.00 (-0.023 to 0.23)	-0.18 (-0.59 to 0.24)	0.14 (-0.30 to 0.58)	0.08 (-0.32 to 0.49)	0.32 (-0.03 to 0.67)	0.21 (-0.13 to 0.56)
Insulin (log ₂) U/ml	0.07 (-0.05 to 0.20)	-0.10 (-0.33 to 0.13)	0.19 (-0.06 to 0.43)	0.06 (-0.16 to 0.28)	-0.01 (-0.20 to 0.19)	0.02 (-0.18 to 0.22)
Glucose (log ₂) mmol/l	-0.80 (-4.29 to 2.69)	0.28 (-6.16 to 6.71)	-1.64 (-8.38 to 5.10)	1.19 (-4.95 to 7.32)	1.35 (-4.13 to 6.82)	-0.49 (-6.13 to 5.15)
Triglycerides mmol/l	-0.30 (-0.83 to 0.24)	0.37 (-0.64 to 1.38)	-0.89 (-1.95 to 0.18)	-1.05 (-2.01 to -0.08)*	-0.39 (-1.22 to 0.44)	-0.34 (-1.20 to 0.53)
HDL mmol/l	0.18 (-0.10 to 0.47)	0.20 (-0.32 to 0.72)	0.07 (-0.48 to 0.61)	0.35 (-0.15 to 0.85)	0.26 (-0.18 to 0.69)	0.20 (-0.23 to 0.63)
IGFI (log ₂) ng/ml	0.45 (0.11 to 0.79)*	-0.12 (-0.75 to 0.51)	0.87 (0.22 to 1.53)	0.64 (0.04 to 1.24)*	-0.04 (-0.59 to 0.50)	0.07 (-0.46 to 0.61)
Leptin (log ₂) ng/ml	-0.05 (-0.21 to 0.10)	0.09 (-0.19 to 0.37)	-0.21 (-0.50 to 0.08)	-0.27 (-0.53 to 0.00)*	0.00 (-0.23 to 0.24)	0.12 (-0.11 to 0.35)
Adiponectin (log ₂) ug/ml	0.82 (0.30 to 1.33)**	0.98 (0.05 to 1.92)*	0.18 (-0.81 to 1.17)	0.34 (-0.56 to 1.24)	0.72 (-0.07 to 1.52)	1.00 (0.20 to 1.80)*
IL6 (log ₂) pg/ml	-0.03 (-0.09 to 0.04)	0.06 (-0.05 to 0.18)	-0.09 (-0.21 to 0.03)	-0.09 (-0.19 to 0.02)	-0.05 (-0.14 to 0.04)	-0.09 (-0.18 to 0.00)
TNF alpha (log ₂) pg/ml	-0.20 (-1.22 to 0.81)	1.51 (-0.33 to 3.35)	-1.99 (-3.92 to -0.06)*	-0.46 (-2.23 to 1.31)	-0.58 (-2.13 to 0.97)	0.90 (-0.63 to 2.43)

Table 4.7: Associations between candidate biomarkers and metabolite clusters from cord blood samples with infant anthropometry at 6 months; data from the UPBEAT study (n=209).

Regression coefficients with corresponding 95% confidence intervals presented are adjusted for maternal parity, ethnicity, smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention and mode of early life feeding. Abbreviations; HDL- High density lipoprotein; IGFI- Insulin growth factor I; IL6-Interleukin 6; MUAC-Mid upper arm circumference; NEFA-non-esterified fatty acids; PCA-principal component analysis; SDS- Standard deviation scores; SFT- skinfold thickness; TNFalpha- Tumour Necrosis Factor alpha. * $p < 0.05$; ** $p < 0.001$.

	Catch up growth (n=246)^	Catch down growth (n=246)^^
	Odds ratio (95% CI)	Odds ratio (95% CI)
PCA-Phosphatidylcholines	1.35 (1.04 to 1.75)*	1.03 (0.86 to 1.22)
PCA-NEFAs	1.34 (0.93 to 1.93)	1.07 (0.82 to 1.39)
C-peptide (log ₂) ng/ml	0.50 (0.24 to 1.05)	1.22 (0.58 to 2.58)
Insulin (log ₂) U/ml	0.72 (0.48 to 1.06)	1.10 (0.74 to 1.65)
Glucose (log ₂) mmol/l	0.04 (0.00 to 55.03)	0.00 (0.00 to 14.21)
Triglycerides mmol/l	1.48 (0.29 to 7.62)	0.54 (0.10 to 3.06)
HDL mmol/l	2.21 (0.93 to 5.26)	1.59 (0.67 to 3.76)
IGFI (log ₂) ng/ml	0.40 (0.14 to 1.16)	5.00 (1.62 to 15.21)*
Leptin (log ₂) ng/ml	0.30 (0.17 to 0.52)**	2.26 (1.37 to 3.75)**
Adiponectin (log ₂) ug/ml	0.20 (0.04 to 1.06)	1.32 (0.27 to 6.89)
IL6 (log ₂) pg/ml	0.94 (0.78 to 1.13)	0.95 (0.79 to 1.14)
TNF alpha (log ₂) pg/ml	1.44 (0.06 to 35.12)	0.20 (0.01 to 5.10)

Table 4.8: Associations between candidate biomarkers and metabolite clusters from cord blood samples and early infant growth velocities; data from the UPBEAT study (n=246).

*Odds ratio with corresponding 95% confidence intervals presented are adjusted for maternal parity, ethnicity, and smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention and mode of early life feeding. Abbreviations; HDL- High density lipoprotein; IGFI- Insulin growth factor I; IL6- Interleukin 6; MUAC-Mid upper arm circumference; NEFA-non-esterified fatty acids; PCA- Principal component analysis; SDS- Standard deviation scores; SFT- skinfold thickness; TNFalpha- Tumour Necrosis Factor alpha. *p<0.05; ** p<0.001. ^ Catch up growth defined as an increase ≥ 0.67 SDs in weight-z-scores from birth to 6 months of age using the WHO reference population (WHO and de Onis, 2006). ^^ Catch down growth defined as a decrease ≤ 0.67 SDs in weight-z-scores from birth to 6 months of age using the WHO reference population (WHO and de Onis, 2006).*

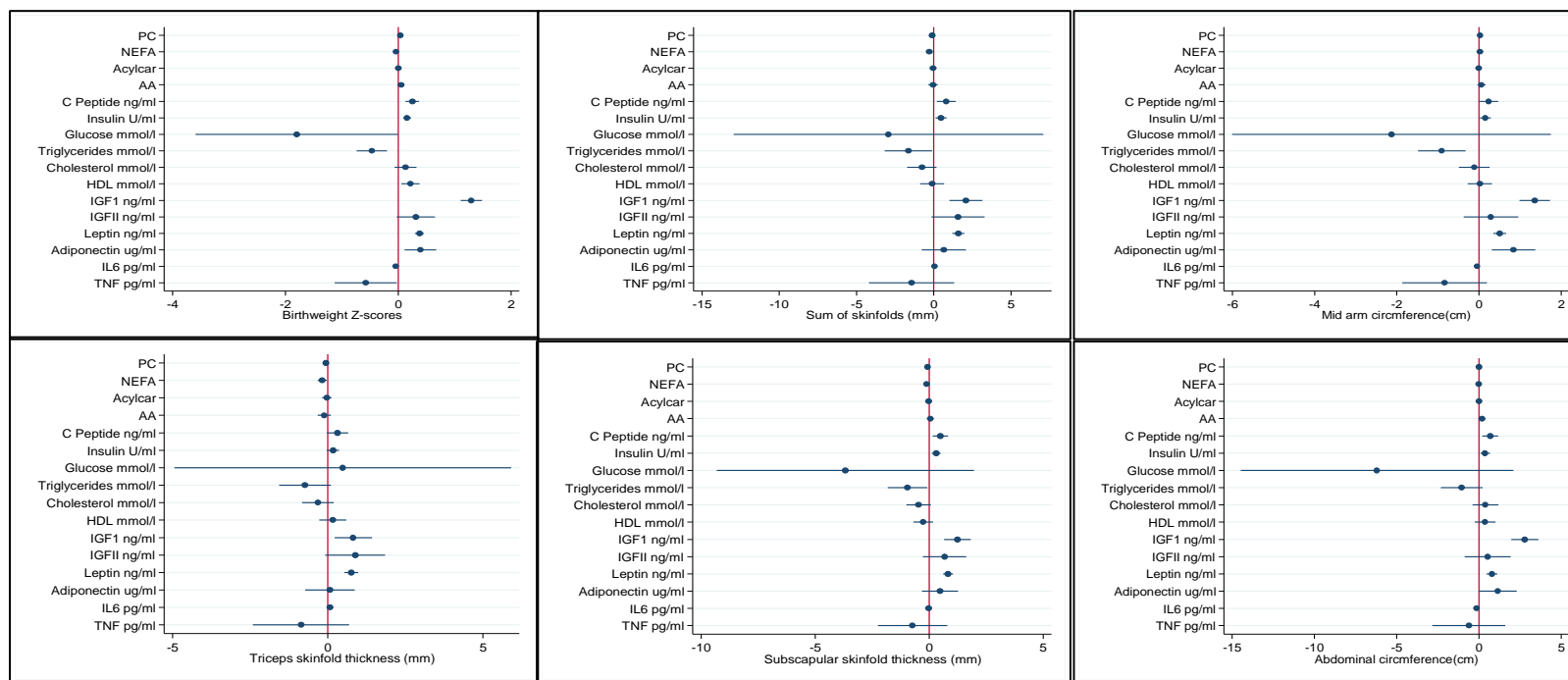


Figure 4.18: Sensitivity analyses following exclusion of offspring born <34 weeks' gestation demonstrating no differences in the associations between cord blood metabolic profile and neonatal anthropometry (excluded n=36).

Regression coefficients graphically presented with corresponding 95% confidence intervals. Adjustment made for maternal parity, ethnicity, and smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention. Abbreviations; AA-amino acids cluster; Acylcar- Long chain acylcarnitines and TCA metabolites cluster; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IGFII- Insulin growth factor II; IL6-Interleukin 6; NEFA-Non-esterified fatty acid cluster; PC-phosphatidylcholine cluster; SFT- skinfold thickness; TNFalpha- Tumour Necrosis Factor alpha

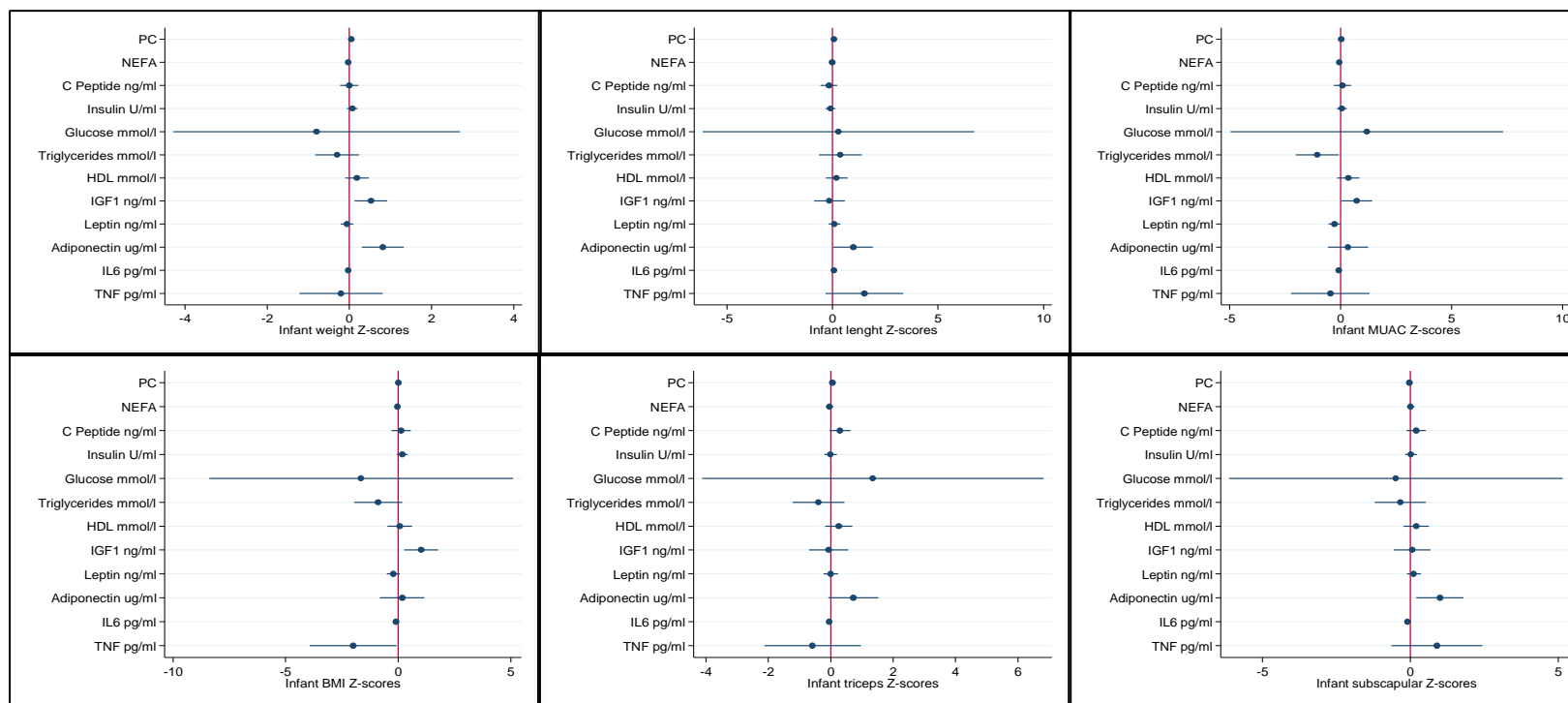


Figure 4.19: Sensitivity analyses following exclusion of offspring born <34 weeks' gestation demonstrating no differences in the associations between cord blood metabolic profile and infant anthropometry at 6 months of age (excluded n=36).

Regression coefficients with corresponding 95% confidence intervals presented are adjusted for maternal parity, ethnicity, smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention and mode of early life feeding. Abbreviations; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IL6-Interleukin 6; NEFA-Non-esterified fatty acid cluster; PC-phosphatidylcholine cluster; SFT- skinfold thickness; TNFalpha- Tumour Necrosis Factor alpha.

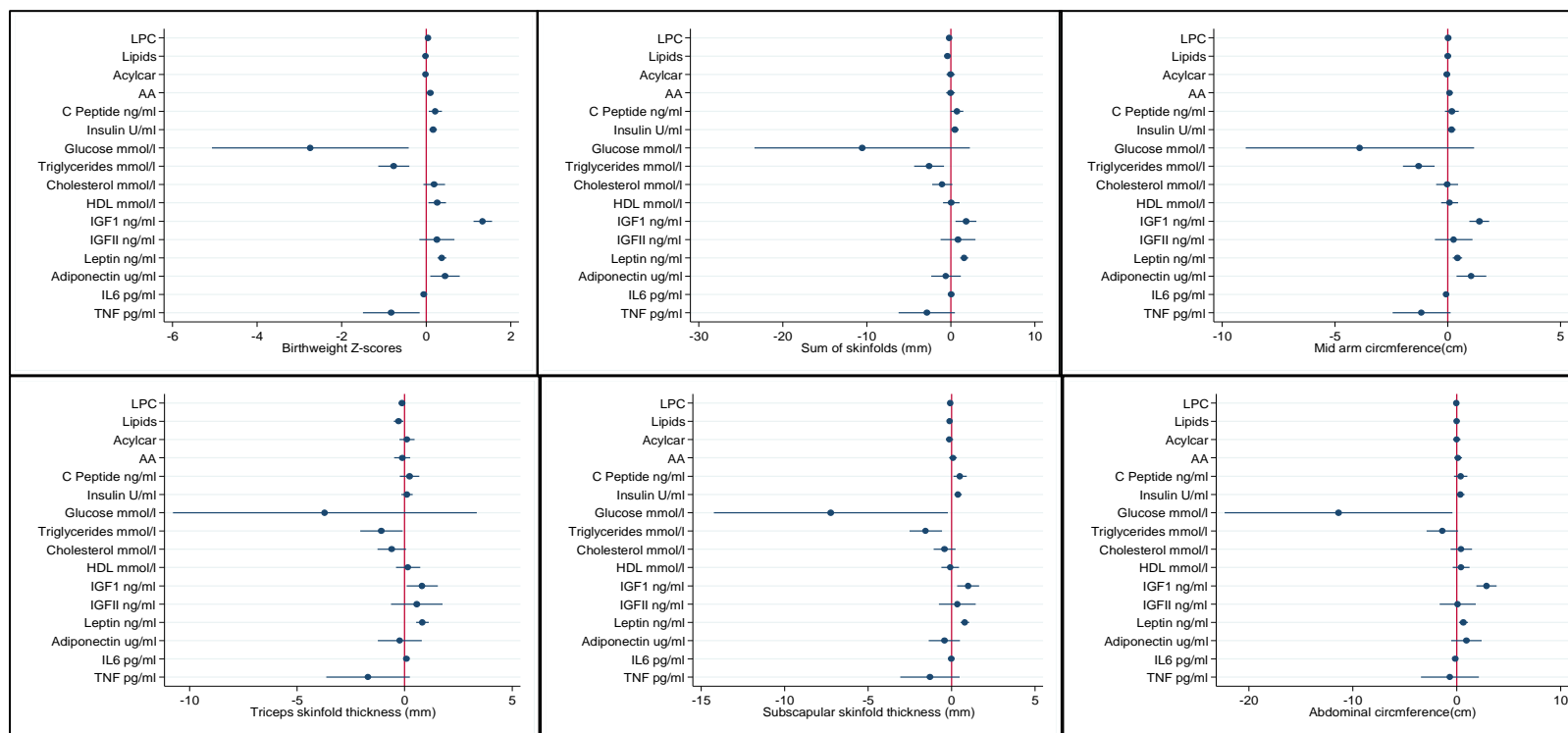


Figure 4.20: Sensitivity analyses of associations between cord blood metabolic profile and neonatal anthropometry, following exclusion of neonates exposed to gestational diabetes *in-utero* excluded n=111).

Regression coefficients graphically presented with corresponding 95% confidence intervals. Adjustment made for maternal parity, ethnicity, and smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention. Abbreviations; AA-amino acids cluster; Acylcar- Long chain acylcarnitines and TCA metabolite clusters; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IGFII- Insulin growth factor II; IL6-Interleukin 6; NEFA-Non-esterified fatty acid cluster; PC-phosphatidylcholine cluster; TNFalpha- Tumour Necrosis Factor alpha

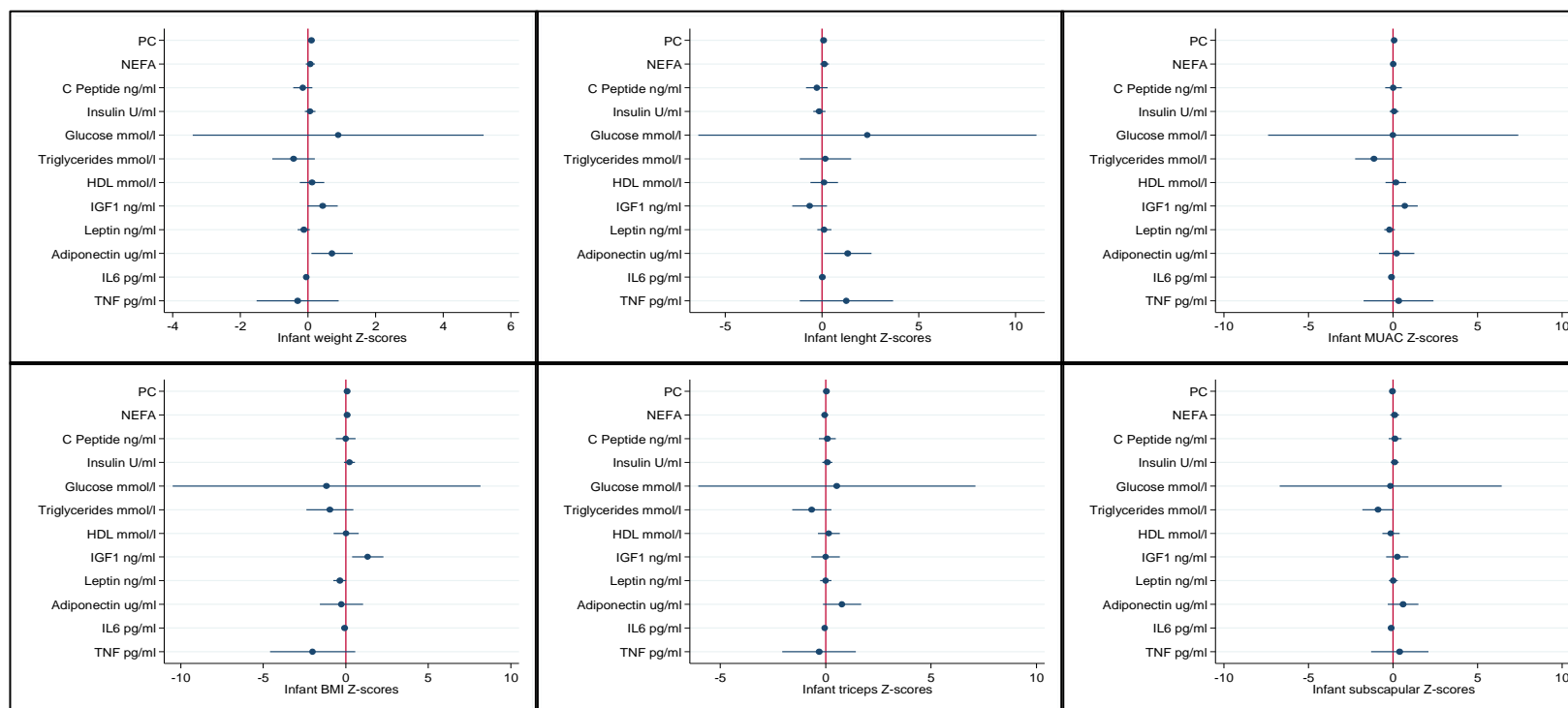


Figure 4.21: Sensitivity analyses of associations between cord blood metabolic profile and infant anthropometry, following exclusion of infants at 6 months exposed to gestational diabetes in-utero (excluded n=111).

Regression coefficients with corresponding 95% confidence intervals presented are adjusted for maternal parity, ethnicity, smoker in early pregnancy, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention and mode of early life feeding. Abbreviations; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IL6-Interleukin 6; NEFA-Non-esterified fatty acid cluster; PC-phosphatidylcholine cluster; TNFalpha- Tumour Necrosis Factor alpha.

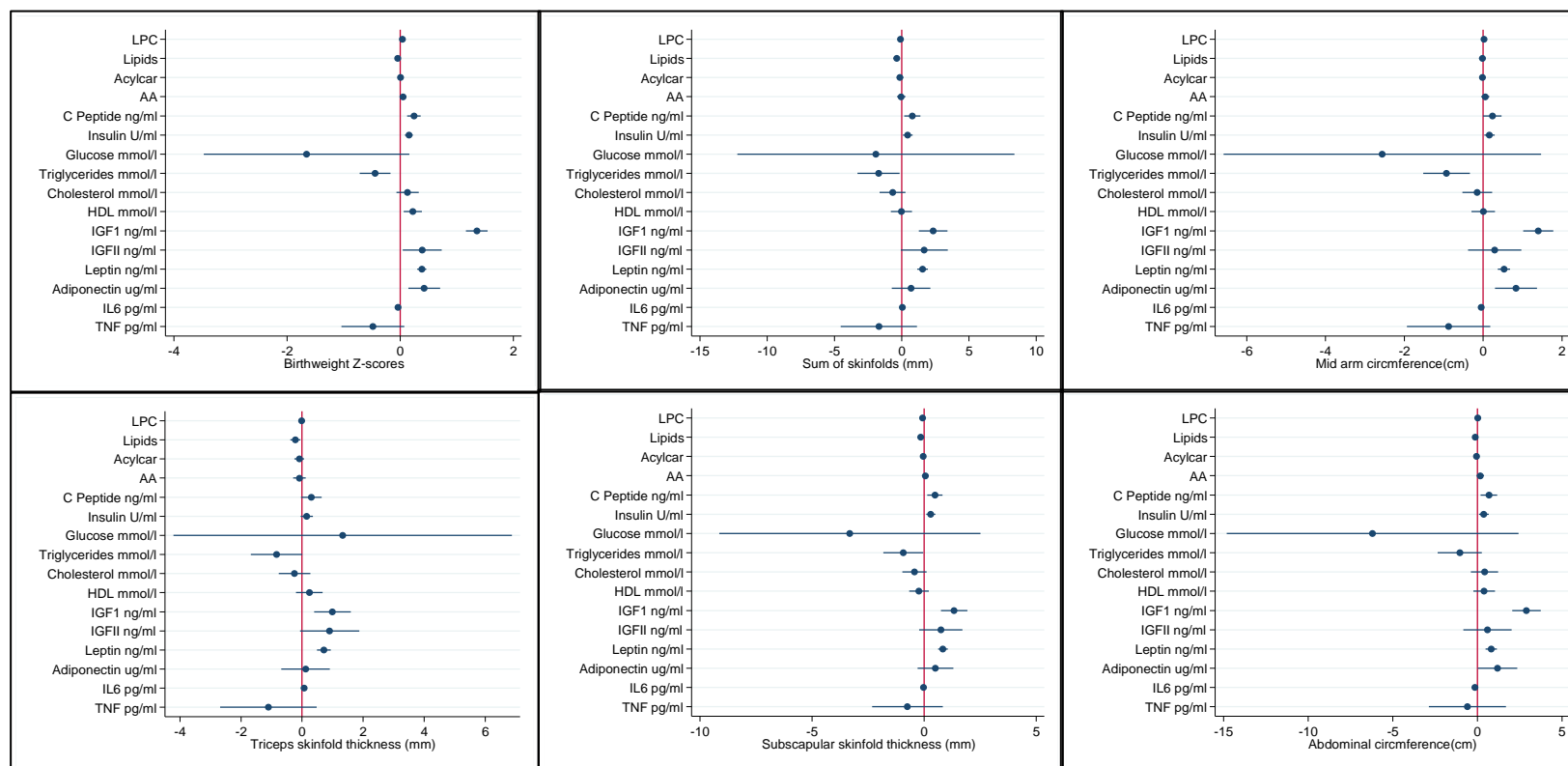


Figure 4.22: Sensitivity analyses of associations between cord blood metabolic profile and neonatal anthropometry, following further adjustment for mode of delivery.

Regression coefficients graphically presented with corresponding 95% confidence intervals. Adjustment made for maternal parity, ethnicity, and smoker in early pregnancy, gestational diabetes, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention and mode of delivery. Abbreviations; AA-amino acids cluster; Acylcar- Long chain acylcarnitines and TCA metabolites cluster; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IGFII- Insulin growth factor II; IL6-Interleukin 6; MUAC-Mid upper arm circumference; NEFA-Non-esterified fatty acid cluster; PC-phosphatidylcholine cluster; SDS- Standard deviation scores; SFT- skinfold thickness; TNFalpha- Tumour Necrosis Factor alpha

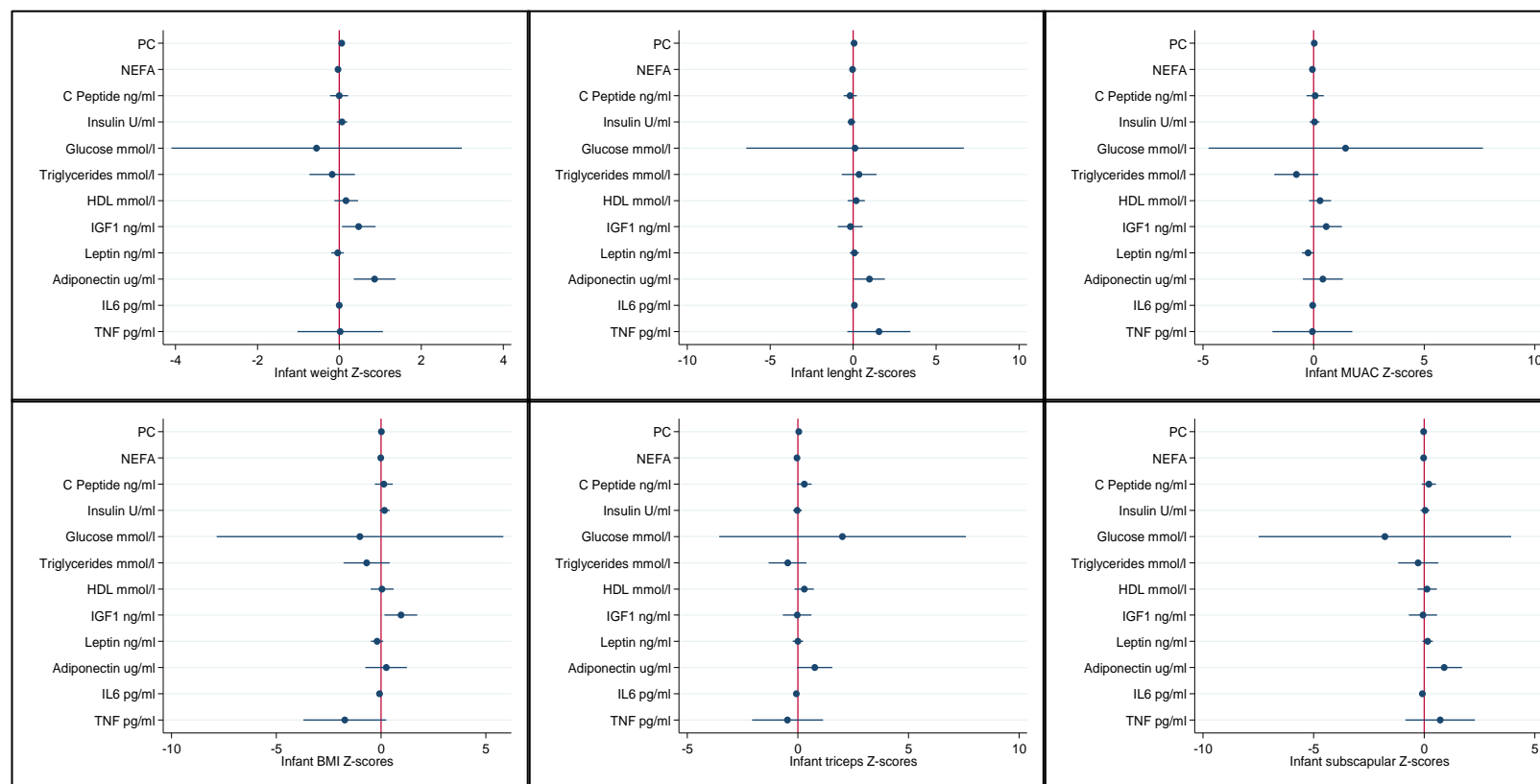


Figure 4.23: Sensitivity analyses of associations between cord blood metabolic profile and infant anthropometry, following adjustment for mode of delivery.

Regression coefficients with corresponding 95% confidence intervals presented are adjusted for maternal parity, ethnicity, smoker in early pregnancy, gestational weight gain, offspring sex, gestation at delivery, randomisation to UPBEAT Intervention, mode of early life feeding and mode of delivery. Abbreviations; HDL- High density lipoprotein; IGF1- Insulin growth factor I; IL6- Interleukin 6; MUAC-Mid upper arm circumference; NEFA-Non-esterified fatty acid cluster; PC-phosphatidylcholine cluster; TNFalpha- Tumour Necrosis Factor alpha.

4.4 Discussion

4.4.1 Summary of overall findings

- There was no effect of the UPBEAT lifestyle intervention on the cord blood metabolic profile.
- A novel relationship between cord lysophosphatidylcholines with neonatal and infant anthropometry was identified which was positively associated with maternal fasting glucose measured in the late second trimester.
- A positive association was observed between cord blood IGF-1 with neonatal measures of growth and body composition, infant weight and mid upper arm circumference z-scores at 6 months.
- An inverse association was identified between cord inflammatory cytokines and measures of infant anthropometry at 6 months.
- There was no relationship between cord triglycerides or NEFAs with measures of neonatal or infant anthropometry at 6 months of age.

This study reports a comprehensive cord blood metabolic profile, including candidate biochemical markers and metabolome, in offspring born to obese mothers. By demonstrating associations with fasting glucose, it has been shown that *in-utero* exposure to maternal dysglycaemia within obese pregnancies, has the potential to modify the cord blood metabolic profile at birth. Although there was no effect of the UPBEAT antenatal lifestyle intervention on the cord blood metabolic profile, when treating the data as a cohort, associations were observed between the cord blood metabolic profile and offspring growth in early life. The novel associations between cord lysophosphatidylcholines, neonatal adiposity together with the relation with maternal hyperglycaemia may provide mechanistic insight in the early-life origins of obesity.

4.4.2 Cord blood lysophosphatidylcholines

Clusters of phosphatidylcholines and lysophosphatidylcholines were found to be positively associated with early growth velocities and weight z-scores, providing possible mechanistic insight of the mechanisms contributing to early postnatal growth in offspring born to obese women. The finding that clusters of cord lysophosphatidylcholines, primarily LPC 16.1 and LPC 18.1 were not only associated with neonatal weight-z-scores, but also infant growth and catch up growth within the first 6 months of age provides novel evidence, suggesting a role in the early life growth velocities. Of relevance, associations with cord lysophosphatidylcholines and birthweight were recently reported in a birth cohort from Germany (Taveras *et al.*, 2004). In this study lysophosphatidylcholines were associated with neonatal adiposity as well as birthweight; suggesting a role in body fat accretion. The observations that lysophosphatidylcholines were associated with infant weight at 6 months could suggest a persistent effect on growth which may have implications for later life obesity. It may be of relevance to these observations that the infant growth trajectory in the first 6 months has been shown to be predictive of adolescence and early adulthood obesity (Ong and Loos, 2006; Taveras *et al.*, 2009; Breij *et al.*, 2014) and that The European Childhood Obesity Programme has shown that lysophosphatidylcholines 14.0 correlate with rapid growth in infancy and subsequent obesity at 6 years of age (Rzehak *et al.*, 2014). Together these findings would suggest a possible role for lysophosphatidylcholines in the early life ‘programming’ of obesity risk (Catalano and Ehrenberg, 2006; Leddy *et al.*, 2008).

Lysophosphatidylcholines are derived from phosphatidylcholines following removal of fatty acids, facilitated predominately by lecithin cholesterol acyltransferase (Jonas, 2000), but also by endothelial lipase or phospholipases. Lysophosphatidylcholines are bound to albumin in human plasma (Belgacem *et al.*, 2007). Further interrogation of the dataset demonstrated a significant linear relationship between cord blood insulin, C-peptide and

IGF-1 with cord lysophosphatidylcholines (Appendix Figure 1-2) supporting the suggestion of an interaction between fetal glucose homeostasis and these molecules (Haggarty, 2010). This observation is in part supported by one small case-control study (n=46), that identified an inverse relationship between maternal gestational diabetes and placental uptake of lysophosphatidylcholines 22:6, in women of heterogeneous BMI (Prieto-Sanchez *et al.*, 2016). A role in fetal metabolism for lysophosphatidylcholines has also been suggested in the non-pregnant state with the development of visceral fat obesity, unrelated to genetic origin but associated with nutritional status (Agras *et al.*, 1987; Pietiläinen *et al.*, 2007; Lee *et al.*, 2015).

A study from the USA, Project Viva cohort, demonstrated that associations between cord blood metabolites from a metabolome, particularly those related to one-carbon metabolism may contribute to rapid postnatal weight gain in offspring born to women of heterogeneous BMI (Isganaitis *et al.*, 2015). Taken together, studies of the cord blood metabolic profile suggest that obesity risk may be determined at birth (Giles *et al.*, 2015). Antenatal interventions directed towards optimising adverse fetal exposures may contribute to curbing the incidence of childhood obesity.

4.4.3 Cord blood insulin growth factor I, adiponectin, leptin and offspring growth

The positive associations between cord blood IGF-1 with neonatal measures of growth and body composition together with infant weight and mid upper arm circumference z-scores at 6 months also suggests a persistent influence of *in-utero* exposures on early growth. In early postnatal life, IGF-1 mediates the effect of growth hormone on early growth (Soubry *et al.*, 2013). Whilst the relationship between the cord blood metabolic profile and differential growth in early infancy suggests a potential persistent effect on growth at 6 months of age, mechanisms must remain conjectural and causal inference should be made with caution. However, several studies have suggested that the IGF-1 gene may be prone

to epigenetic modification *in-utero* (Baker *et al.*, 1993; Kao *et al.*, 1994; Fu *et al.*, 2009) with animal studies shedding some light on this, providing evidence of an interaction with maternal glycaemia status. For example, Zinkhan *et al.*, demonstrated that *in-utero* exposure to maternal glycaemia in rats led to decreased hepatic H3Me3K36 and mRNA variants of the IGF-1 gene in the offspring (Zinkhan *et al.*, 2012). Others have implicated a role of these variants to a predisposition to later obesity and insulin resistance (Selak *et al.*, 2003; Fu *et al.*, 2009). Whether epigenetic modification may also influence lipid metabolism including that of lysophosphatidylcholines, remains conjectural.

The linear associations with cord adiponectin and measures of weight, length and subscapular z-scores at 6 months are in keeping with recent evidence from a prospective cohort study from Germany, in children born to women of heterogeneous BMI (n=141); suggesting a potential long term influence in children at 5 years of age (Meyer *et al.*, 2017). Cord blood leptin was associated with measures of neonatal growth and body composition and increased odds of catch up growth from birth to 6 months of age, suggesting a potential mediatory role of early infancy growth. Cord blood leptin has been implicated as a proxy for neonatal fat mass as it is synthesised by the adipocyte Ob gene and is proportional to adipose tissue mass (Zimmet *et al.*, 1996; Lawlor *et al.*, 2014). This study has demonstrated an inverse relationship with cord leptin and catch up growth independent of birthweight which may be explainable by a state of leptin resistance in early infancy, as observed in previous studies. Studies have demonstrated an inverse relationship with cord blood leptin and catch up growth independent of birthweight, which may be explainable by a state of leptin resistance in early infancy (Ong *et al.*, 1999; ní Chaoimh *et al.*, 2016).

4.4.4 *Relationship between maternal gestational diabetes and cord blood metabolic profile*

The associations between maternal gestational diabetes, specifically fasting glucose, at the time of the oral glucose tolerance test with the cord blood metabolic profile, adds to the previously documented relationships including observations from the HAPO study, documenting linear associations of maternal glycaemic profile with cord blood C-peptide and adiponectin (Metzger *et al.*, 2008; Lowe *et al.*, 2010).

The linear associations between cord blood anabolic hormones including cord blood C-peptide, insulin and IGF-1 with measures of growth and body composition at birth, agree with previous studies in offspring born to women of heterogeneous BMI (Wang *et al.*, 1991; Christou *et al.*, 2001; Delvaux *et al.*, 2003; Carlsen *et al.*, 2015) and concur with the knowledge that insulin and IGF-1 are the most important regulators of fetal growth in the 2nd and 3rd trimester (Christou *et al.*, 2001; Catalano *et al.*, 2009). IGF-1 has been shown consistently to be raised in cord blood of offspring born to obese women, predominately as a consequence of maternal dysglycaemia (Metzger *et al.*, 2009; Harmon *et al.*, 2011; Lawlor *et al.*, 2014) .

4.4.5 *Cord blood lipids and inflammatory mediators and offspring anthropometry*

Triglycerides and non-esterified fatty acids in maternal circulation have been widely implicated as determinants of fetal growth in obese and diabetic women (Catalano *et al.*, 2009). However, in this study, an inverse relationship between clusters of non-esterified fatty acids and triglycerides with neonatal growth and adiposity was observed, which has also been reported by others (Jones *et al.*, 1999; Rodie *et al.*, 2004; Kelishadi *et al.*, 2007; Cekmez *et al.*, 2009). This association with low rather than high birth weight may reflect mobilisation of lipids as an alternative fuel source (Jones *et al.*, 1999; Kelishadi *et al.*,

2007). Importantly, this study questions the role of triglycerides in the determination of neonatal adiposity.

Despite the suggestion that inflammatory mediators (interleukin-6 and tumour necrosis factor- α) may contribute to the development of neonatal adiposity *in-utero*, through regulation of central pathways of satiety and appetite, this study reported an inverse association with neonatal body composition. There is no obvious explanation for this finding (Yessoufou and Moutairou, 2011; Cesar and Pisani, 2016).

4.4.6 *Strengths and Limitations*

Strengths of this study include an extensive assessment of the cord blood metabolic profile at birth, detailed neonatal and infant anthropometric data collection and prospective collection of maternal early pregnancy BMI, total gestational weight gain and measures of maternal insulin resistance. Using data reduction techniques for the cord blood metabolome, metabolite clusters of biological importance associated with measures of neonatal and infant anthropometry were identified.

Limitations include the collection of mixed cord blood (umbilical artery and vein), which weakens conclusions regarding fetal or maternal origin of the metabolites in previously published reports as well as this study (Taveras *et al.*, 2004; Isganaitis *et al.*, 2015). Whilst treatment of GDM has the potential to influence cord insulin, C-peptide and IGF-1 concentrations, this was not adjusted for within this analysis (Landon *et al.*, 2015).

However, a sensitivity analysis, removing women with GDM, did not modify the observed relationships. Duration of labour has been previously shown to influence cord blood metabolic profile, but this data was not available, however mode of delivery did not modify the observed associations (Hashimoto *et al.*, 2013; Logan *et al.*, 2016a). It must be recognised that the metabolome and the candidate markers measured provide only an

incomplete profile of the late pregnancy *in-utero* fetal exposures as unmeasured micronutrients, essential fatty acids and steroid hormones may also contribute to neonatal and early life growth and body composition.

4.4.7 Conclusion

This study, of more than 300 infants describes for the first time a comprehensive cord blood metabolic profile in offspring born to obese women. Known associations of metabolic variables with infant adiposity were confirmed and questions raised regarding previous associations derived from smaller cohorts. Importantly this study has highlighted novel associations with lipid sub-species and early postnatal growth, and provides supporting evidence that IGF-1 at birth may be a determinant of later growth trajectories. Despite the lack of difference in metabolites in control and intervention arms, this thesis has reported a difference in infant adiposity in 6 month old children (Patel *et al.*, 2017). Current investigation of the maternal metabolome and neonatal epigenome may shed light on the causative mechanisms and further insight into growth trajectories. Ongoing studies of the cord epigenome may provide further mechanistic insight into potential pathways. Replication in other cohorts including the use of Mendelian randomisation methods are required to determine causality. Ongoing follow-up of the UPBEAT offspring will address the long-term implications of these observed associations.

Chapter 5 Infant adiposity following a randomised controlled trial of a behavioural intervention in obese pregnancy.

5.1 Background

The high prevalence of childhood obesity is a major health concern, with 27.3% of children estimated to be overweight or obese in the USA (Cunningham *et al.*, 2014). A combination of antenatal and postnatal exposures including environmental factors have been implicated in the development of childhood obesity (Nelson *et al.*, 2010; Young *et al.*, 2012), which has been shown to track into adulthood (Cunningham *et al.*, 2014). Observational studies suggest that manipulation of maternal metabolism through diet and/or physical activity in the antenatal period has the potential to reduce childhood obesity (Okubo *et al.*, 2014) which has been unequivocally achieved in pregnant obese experimental animals and their offspring (Patel *et al.*, 2015). These observations have led to a consensus that obesity is in part ‘programmed’ *in-utero*, in keeping with the ‘developmental programming’ hypothesis (Patel *et al.*, 2015). As mentioned in *Chapter 1: Introduction*; well-designed RCTs in pregnant women and their offspring are required to infer causality through minimising selection bias and confounding (Richmond *et al.*, 2014; Patel *et al.*, 2015).

The UPBEAT of a dietary and physical activity intervention in 1555 obese pregnant women, where women were randomised to standard antenatal care or standard antenatal care with an intense behavioural intervention that focussed on improving insulin sensitivity through reducing dietary glycemic load and saturated fat intake (Poston *et al.*, 2015) (Further details provided in *Chapter 2:Methods*). Although the intervention did not reduce GDM or LGA delivery (maternal and neonatal primary outcomes), there were significant improvements in maternal antenatal diet and body composition (Poston *et al.*, 2015), all of which have been implicated with the development of childhood obesity.

To examine the hypothesis that the lifestyle intervention might reduce the influence of maternal obesity on offspring adiposity, our principal aim was to assess whether the UPBEAT intervention was associated with a reduction in measures of infant adiposity at 6 months of age, a pre-defined hypothesis within the trial protocol (Briley *et al.*, 2014). Assessment was made to identify whether the pregnancy intervention had lasting influence on maternal diet and physical activity, and on known early-life determinants of infant adiposity, including breastfeeding.

5.2 Research design and methods

5.2.1 Study design and setting

Between July 2010 and May 2015, a planned follow up at 6 months postpartum was undertaken of mothers and their offspring who had participated in the UPBEAT RCT in eight inner-city NHS Trust Hospitals in the UK. Further details of the UPBEAT study design and participating centres are provided in *Chapter 2: Methods*.

5.2.2 Study population and consent

1555 women were recruited to the UPBEAT trial (≥ 16 years of age; pre-pregnancy BMI ≥ 30 kg/m²). Further details of the UPBEAT's exclusion and inclusion criteria are provided in *Chapter 2: Methods*. If a participant had withdrawn from the trial but was willing to take part ($n=2$), written consent was obtained at the 6 month visit. Infants were excluded if aged ≤ 4 months or ≥ 8 months of age at this visit. Comparison of demographic details at trial entry was made between women who declined to participate and those who took part.

5.2.3 Outcomes

5.2.3.1 Infant anthropometry

The principal outcome of interest was infant adiposity assessed by measurement of infant SFT (triceps and subscapular, measured in triplicate by trained research staff using infant skinfold callipers). Subsidiary infant outcomes of infant adiposity included SSFT, estimated total body fat; weight, abdominal and upper mid-arm circumferences (Slaughter *et al.*, 1988). For these measures, where reference WHO population data were available, z-scores were calculated (WHO and de Onis, 2006), including adjustment for infant age and sex. Occipitofrontal circumference, and crown-rump length and crown-heel length obtained with a calibrated infantometer were also measured. Further details regarding measurement methodology and data management are provided in *Chapter 2: Methods*.

Duration of breastfeeding, weaning history, measures of appetite, infant sleeping patterns, physical activity, healthcare resource use and childcare (Briley *et al.*, 2014) were pre-specified outcomes. These were evaluated using the Infant Feeding and Growth Questionnaire (Robinson *et al.*, 2007), the Baby Eating and Behaviour Questionnaire (Llewellyn *et al.*, 2011), the BISQ (Brief Infant Sleep Questionnaire) (Sadeh, 2004), the Infant Behaviour Questionnaire (for infant temperament)(Gartstein and Rothbart, 2003) and questionnaires ascertaining infant health, medical resource use and early care and education, respectively. Further details of questionnaires and respective data management procedures are provided in *Chapter 2: Methods*.

5.2.3.2 Maternal dietary and physical activity analysis

As discussed in *Chapter 2: Methods*, maternal diet at 6 months postpartum was assessed using the same semi-quantitative FFQ and analysed as previously reported for the mothers during their pregnancy (Poston *et al.*, 2015). Data was analysed only in questionnaires which were fully completed for both maternal diet and physical activity. Those with incomplete/missing dietary data were excluded (65.8%). There was no missing physical activity data. The main outcomes of interest were maternal dietary glycaemic load, saturated fat intake and energy intake. Other outcomes included glycaemic index, protein and fibre intake. Physical activity was assessed, as it had been in pregnancy, using the IPAQ and summarised as METs of energy expenditure (IPAQ, 2005). Further details regarding data management procedures for both maternal dietary and physical activity analyses are provided in *Chapter 2: Methods*.

5.2.4 Statistical analysis

A complete-case analysis was undertaken for all participating mothers and infants. Treatment effects for continuous outcomes were expressed as differences in means obtained from multivariable linear regression, and binary endpoints as risk ratios with 95% confidence obtained using binomial regression. For both, adjustment was made for minimisation variables (maternal BMI at trial enrolment, parity and ethnicity), infant sex and age at follow up. The number of intervention contact sessions during pregnancy were evaluated in relation to measures of infant adiposity.

Although loss to follow-up was similar in both trial arms, assessment was made for the possibility that loss to follow-up resulted in selection bias using three complementary methods. All sets of analyses were pre-planned sensitivity analyses. Using Little's chi-squared covariate dependent missing (CDM) test to explore evidence of the data being MNAR (i.e. the possibility that those who were lost to follow-up the effect of the intervention on outcome differed from those who did attend the follow up) for both offspring and maternal outcomes was undertaken (Catalano and Hauguel-De Mouzon, 2011).

Second, for the primary offspring outcomes only (subscapular and triceps SFT), several simulation datasets were generated, over a range of scenarios regarding missing data in both arms of the study that were informed by predictors of loss to follow-up (maternal BMI at trial enrolment, parity and ethnicity) (White *et al.*, 2011). The scenarios selected aimed to cover a range of plausible situations that could result in bias under the assumption of data being MAR. The analysis was undertaken using the STATA command 'rctmiss' for the primary outcome of the study (Patro-Golał *et al.*, 2016b). For the purpose of this sensitivity analysis δ was assumed as .5, .75, 1, 1.5, 2. Thirdly, for the primary infant outcomes only, multivariate imputation by chained equations was used to impute missing

data for infant adiposity to investigate whether loss to follow up may have resulted in type 2 statistical error. Data was imputed to create 50 datasets using 10 burn-in iterations for live-born infants using maternal and infant variables throughout pregnancy and early postpartum period.

Pre-defined interaction analyses using Wald tests were performed for duration of exclusive breast feeding (< 3 months vs. ≥ 3 months) and neonatal sex. Statistical significance for interaction was determined as $p \leq 0.05$. As the intervention was associated with modifications in maternal diet, assessment of the effect of maternal dietary glycaemic load, saturated fat and energy intake at 27-28⁺⁶ weeks' gestation and GWG on any differences observed in infant skinfold thicknesses using the method of average causal mediation analysis were undertaken (Hicks and Tingley, 2011). Similarly, assessment was made for any differences observed in maternal diet at 6 months postpartum on any observed differences in skinfold thicknesses adjusted for maternal antenatal diet.

5.3 Results

5.3.1 Demography

Of the 1555 participants randomised to UPBEAT at 15-18⁺⁶ week's gestation between July 2010 and May 2015 and with a live born infant, 1522 were approached between 4-8 months postpartum to attend a 6-month follow up. Of these 1522, 720 (47.3%) infants and 707 (46.5%) mothers took part in this study (Figure 5.1). Thirteen mothers were excluded as they were pregnant at time of study, and 22 infants were excluded because the follow up appointment was held ≤ 4 months or ≥ 8 months of age (Figure 5.1). In comparison to those who did not take part, mothers who attended the 6 month visit were on average 1.3 years older, more likely to be Caucasian, nulliparous, to have had GDM in the index pregnancy (28.2% vs. 23.3%; $p=0.041$), and were less likely to be current smokers (Table 5.1). There were no differences in maternal early pregnancy BMI and SSFT between women who participated in the 6 month follow-up visit compared to those who did not. Women in the intervention arm demonstrated reduced GWG as previously reported (Poston *et al.*, 2015). The infants who attended the 6 month appointment had a greater gestational age at delivery (by 2 days), were 67g heavier, and more likely to have been breastfed at birth than those that did not attend the follow up visit (Table 5.2).

There was no difference between mean maternal BMI between the intervention and standard care groups at trial entry (36.17 vs. 36.31 kg/m², respectively) or at 6 months postpartum (36.26 vs. 36.45 kg/m², respectively). The incidence of maternal smoking at 15-18⁺⁶ weeks' gestation was higher in the standard antenatal care arm in comparison to the intervention arm (5.6% vs. 2.0%) (Table 5.3). There were no differences in all other demographic and clinical variables between the two study arms (Table 5.3).

5.3.2 *Infant anthropometry*

Three hundred and fifty six infants in the standard antenatal care arm and 342 infants in the intervention arm (mean age 5.82 months) had anthropometric measurements collected at 6 months of age. There was no statistical difference in triceps SFT in the intervention vs. the standard care arm (difference -0.14 SD, 95% CI -0.38 to 0.10), $p=0.246$), but subscapular SFT z-score was -0.26 SD (-0.49 to -0.02; $p=0.03$) lower in the intervention arm (Table 5.4). Infants in the intervention arm had a 5% lower subscapular SFT (-0.38mm; -0.70 to -0.06; $p=0.02$), compared to infants in the standard antenatal care arm (Table 5.4). The infant SSFT was 0.63mm lower in the intervention arm, but did not reach statistical significance ($p=0.06$) in comparison to the standard antenatal care arm (Table 5.4). There were no differences in BMI z-score and abdominal circumference (Table 5.4) or in other anthropometric measures between the two arms (Table 5.4).

5.3.2.1 Sensitivity analyses

Maternal smoking status at trial entry, did not influence the difference in subscapular SFT between the two arms (Table 5.5), as with the use of multiple imputation (Table 5.6). There was no evidence for trial outcomes being missing anything more than completely at random ($p = 0.856$). Undertaking sensitivity analyses for deviation from the missing at random assumption, significant differences in infant subscapular SFT (mm) were found within a range of -0.35 to -0.38mm dependent on the assumption of missingness taken (Table 5.7). Similar results to the complete-case analysis were also observed for infant triceps skinfold thickness (Table 5.8).

5.3.3 *Infant early life feeding, appetite, satiety and general health at 6 months*

There was no difference in infant feeding between the two trial arms, nor appetite, satiety responsiveness and infant childcare. Infants were exclusively breastfed, on average for 82.7

(SD 65.3) days and total number of hours spent sleeping were similar between arms (Table 5.9). There was an increase in infant inpatient nights in the intervention arm, attributable to 1 infant requiring long-term hospital admission due a ventricular septal defect repair (Table 5.9). No differences in infant use of medications or in the cause of hospital inpatient admissions, except for gastrointestinal related disorders, which were lower in the intervention arm were observed (Table 5.10). There was no association between the number of antenatal contact sessions with the health trainer and measures of infant anthropometry (Table 5.11).

5.3.4 Pre-defined interaction tests

No interactions were observed between randomisation allocation and infant sex (Table 5.12), but there was a significant interaction of breast feeding ($< 3\text{mths}/\geq 3\text{mths}$) with the intervention; triceps skin fold thickness was lower in infants of mothers in the intervention arm who breastfed ≥ 3 months *vs* those in the standard care arm -0.90mm (-1.59 to -0.21); $p=0.01$; Wald interaction test; $p=0.02$) (Figure 5.2). Similar patterns of differences of effect by breastfeeding for SSFT, estimated total body fat and arm circumference did not achieve statistical significance (p -values for interactions all ≥ 0.05) (Table 5.13).

5.3.5 Maternal diet and physical activity at 6 months postpartum

In those women who provided complete dietary data glycaemic index, glycaemic load, saturated fat and total energy intake were reduced in the mothers in the intervention arm in comparison to standard care, as well as a significant reduction in total fat and protein intakes (Table 5.14, Figure 5.3). When the under-reporters (calorie intake) were included in sensitivity analyses, there were no differences in effect size estimates of dietary variables. Furthermore, there was no difference in maternal characteristics (including maternal age, BMI and socioeconomic deprivation status) between those under-reporting and those not

under-reporting calorie intake. There was no effect of the intervention of maternal physical activity (Table 5.14).

5.3.6 *Mediation analysis*

Causal analysis suggested a partial indirect effect of the intervention associated with reduction in maternal early GWG (between 15-18⁺⁶ and 27-28⁺⁶ weeks' gestation) ($p=0.015$), late GWG (between 27-28⁺⁶ and 34-36 weeks' gestation) ($p=0.009$), total GWG ($p=0.014$) and maternal dietary saturated fat intake at 27-28⁺⁶ week's gestation ($p=0.016$) in relation to infant subscapular skinfold thickness at age 6 months (Figure 5.4). In contrast, there was no suggested effect of postnatal maternal diet on the observed differences in infant subscapular skinfold measurements (Figure 5.5). As there was no effect of the intervention on maternal physical activity, there was no rationale for exploring a causal mediating role of maternal physical activity on offspring adiposity.

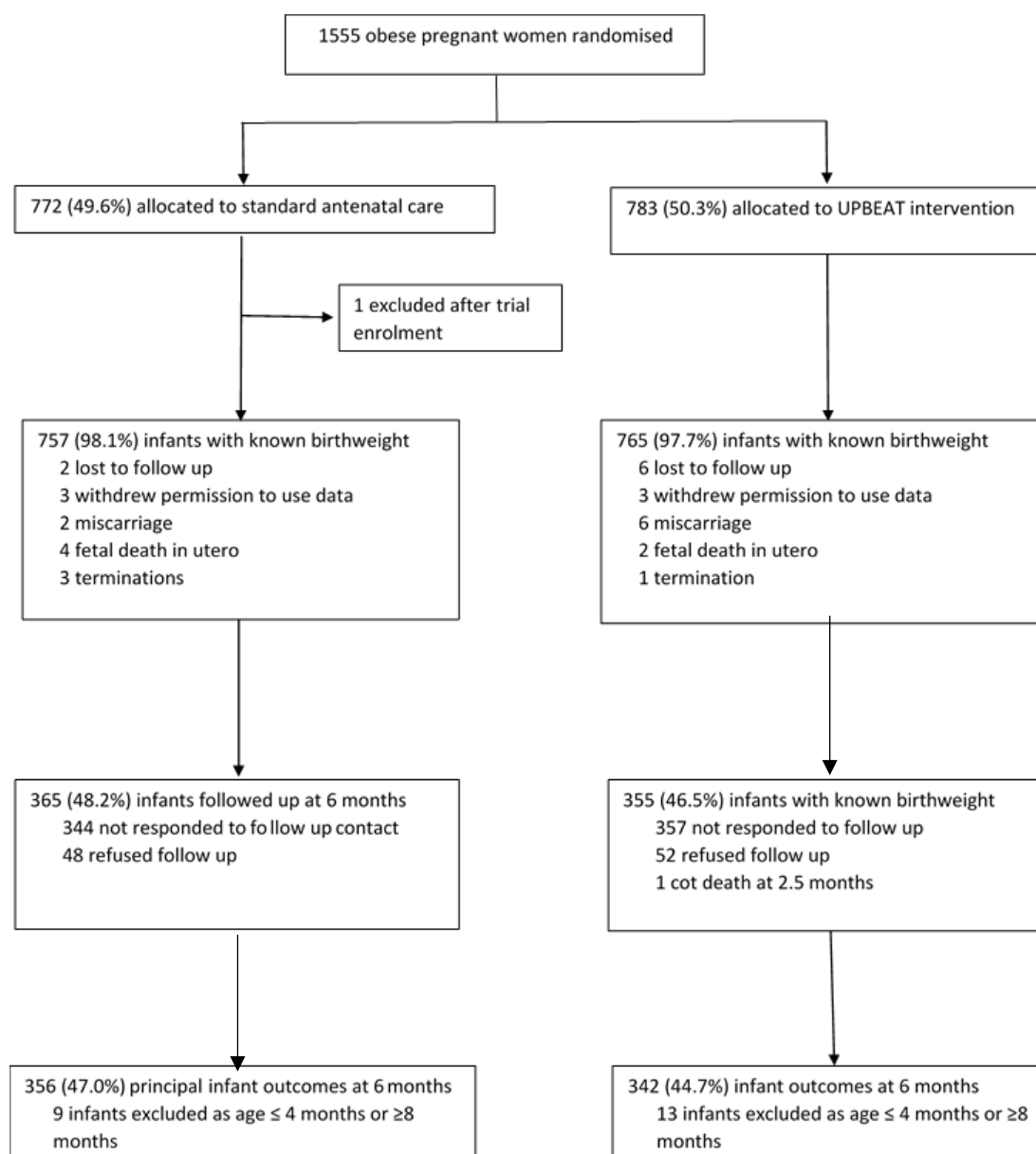


Figure 5.1: Consort diagram of participants enrolled in the UPBEAT trial at 6 months postpartum.

Maternal		Followed up					Not followed up					Follow up vs. no follow-up p-value
		Intervention		Control		Mean difference/ OR (95%CI) *	Intervention		Control		Mean difference/ OR (95% CI) *	
Maternal age (years)		N=355	31.39 (5.09)	N=365	30.96 (5.54)	0.43 (-0.35 to 1.21)	N=407	29.74 (5.68)	N=392	29.94 (5.49)	-0.20 (-0.98 to 0.57)	<0.001
Maternal BMI (kg/m²)		N=355	36.27 (5.06)	N=365	36.36 (4.72)	-0.09 (-0.81 to 0.62)	N=407	36.36 (4.84)	N=392	36.20 (4.50)	0.16 (-0.48 to 0.81)	0.89
Maternal ethnicity	Asian	N=355	14 (3.9)	N=365	11 (3)	1.31 (0.60 to 2.84)	N=407	29 (7.1)	N=392	37 (9.4)	0.75 (0.47 to 1.20)	<0.001
	Black		65 (18.3)		76 (20.8)	0.88 (0.65 to 1.18)		128 (31.4)		118 (30.1)	1.01 (0.85 to 1.29)	<0.001
	White		21 (5.9)		22 (6.0)	0.98 (0.55 to 1.75)		23 (5.7)		18 (4.6)	1.23 (0.67 to 2.24)	<0.001
	Other		255 (71.8)		256 (70.1)	1.02 (0.93 to 1.12)		227 (55.8)		219 (55.9)	1.00 (0.88 to 1.13)	0.47
Multiparous		N=355	176 (49.6)	N=365	178 (48.8)	1.02 (0.88 to 1.18)	N=407	260 (63.9)	N=392	245 (62.5)	1.02 (0.92 to 1.14)	<0.001
Current smoker		N=355	8 (2.3)	N=365	20 (5.5)	0.41 (0.18 to 0.92)	N=407	39 (9.6)	N=392	38 (9.7)	0.99 (0.65 to 1.51)	<0.001
IMD quintiles	1	N=354	16 (4.5)	N=364	19 (5.2)	-	N=403	13 (3.2)	N=392	15 (3.8)	-	0.16
	2		30 (8.5)		19 (5.2)	1.87 (0.78 to 4.52)		26 (6.5)		25 (6.4)	1.20 (0.48 to 3.02)	
	3		37 (10.5)		40 (11.0)	1.10 (0.49 to 2.45)		50 (12.4)		44 (11.2)	1.31 (0.56 to 3.06)	
	4		122 (34.5)		140 (38.5)	1.03 (0.51 to 2.10)		117 (29.0)		138 (35.2)	0.98 (0.45 to 2.14)	
	5		149 (42.1)		146 (40.1)	1.21 (0.60 to 2.45)		197 (48.9)		170 (43.4)	1.34 (0.62 to 2.89)	
Maternal anthropometry	Sum of skin folds (mm)	N=349	124.06 (28.23)	N=363	122.28 (25.49)	1.79 (-2.17 to 5.75)	N=401	121.27 (28.6)	N=386	123.42 (27.22)	-2.15 (-6.06 to 1.76)	0.56
Antenatal history	GDM	N=349	102 (29.2)	N=355	96 (27.0)	1.08 (0.85 to 1.37)	N=287	60 (20.9)	N=306	77 (25.2)	0.83 (0.62 to 1.12)	0.04
	PET	N=352	11 (3.1)	N=362	11 (3.0)	1.03 (0.45 to 2.34)	N=392	16 (4.1)	N=382	16 (4.2)	0.97 (0.49 to 1.92)	0.28
	Total gestational weight gain from pre-pregnancy weight	N=333	6.88 (4.70)	N=341	7.82 (4.42)	-0.93 (-1.62 to -0.24)	N=193	7.71 (4.35)	N=226	7.76 (4.59)	-0.05(-0.91 to 0.81)	0.18

Table 5.1: Maternal demographics of those who consented to 6 months follow up in the UPBEAT trial versus those who did not by randomisation allocation (included n=720 versus excluded n=799).

**Difference calculated for maternal offspring followed up (intervention vs. control) & those not followed up (intervention vs. control) at 6-months postpartum. Abbreviations; GDM-Gestational diabetes, IMD-Index of multiple deprivation; PET-Preeclampsia.*

		Follow up at 6 months		No follow up		Mean Difference/ Odds ratio (95% Confidence Intervals)	p-value
			Mean (SD)/N (%)		Mean (SD)/N (%)		
Birth characteristics	Gestation at birth (weeks)	N=720	39.64 (1.95)	N=799	39.37 (2.54)	0.26 (0.04 to 0.49)	0.02
	Admission to NICU	N=720	56 (7.8)	N=799	66 (8.3)	0.94 (0.67 to 1.32)	0.73
	Breastfeeding at birth	N=717	445 (62.1)	N=776	416 (53.6)	1.16 (1.06 to 1.26)	<0.001
Anthropometry	Birthweight (grams)	N=720	3457.62 (565.18)	N=799	3390.11 (647.71)	67.51 (6.46 to 128.56)	0.03
	Birthweight >4kg	N=720	102 (14.2)	N=799	108 (13.5)	1.05 (0.82 to 1.35)	0.71
	LGA >90 th	N=720	60 (8.3)	N=799	73 (9.1)	0.91 (0.66 to 1.26)	0.58
	SGA <10 th	N=720	84 (11.7)	N=799	92 (11.5)	1.01 (0.77 to 1.34)	0.93
	Subscapular skinfold thickness (mm)	N=354	10.87 (2.64)	N=148	11.06 (2.64)	-0.20 (-0.33 to 0.23)	0.73
	Triceps skinfold thickness (mm)	N=363	5.26 (1.53)	N=154	5.41 (1.46)	-0.15 (-0.43 to 0.13)	0.29
	Sum of skinfold thickness (mm)	N=354	10.87 (2.64)	N=148	11.06 (2.64)	-0.20 (-0.70 to 0.31)	0.73
	Abdominal circumference (cm)	N=386	11.45 (1.10)	N=182	11.54 (1.10)	-0.09 (-0.28 to 0.10)	0.36
	Mid-arm circumference (cm)	N=414	11.45 (1.10)	N=148	11.06 (2.34)	-0.20 (-0.70 to 0.31)	0.44

Table 5.2: Infant characteristics of those who consented to 6 months in the UPBEAT trial follow-up versus those who did not (included n=720 versus excluded n=799).

Abbreviations; LGA-Large for gestational age, NICU-Neonatal intensive care unit, SGA-Small for gestational age

		Intervention (n=342)		Control (n= 356)	
			Mean (SD)/N (%)		Mean (SD)/N (%)
Maternal demographics					
Pre-pregnancy					
Maternal age (years)		N=342	31.30 (5.04)	N=356	31.00 (5.58)
Maternal ethnicity	Asian	N=342	14 (4.1)	N=356	11 (3.1)
	Black		62 (18.1)		72 (20.2)
	Other		19 (5.6)		22 (6.2)
	White		247 (72.2)		251 (70.5)
Multiparous		N=342	169 (49.4)	N=356	174 (48.9)
Index of multiple deprivation quintiles*	1 (least deprived)	N=341	15 (4.4)	N=355	19 (5.4)
	2		29 (8.5)		19 (5.4)
	3		35 (10.3)		38 (10.7)
	4		119 (34.9)		136 (38.3)
	5 (most deprived)		143 (41.9)		143 (40.3)
Family history	GDM	N=333	10 (3.0)	N=344	11 (3.2)
	PET	N=333	40 (12.0)	N=344	33 (9.6)
	T2DM	N=341	86 (25.2)	N=356	70 (19.7)
15-18⁺⁶ weeks' gestation					
Current smoker [^]		N=342	7 (2.0)	N=356	20 (5.6)
Maternal anthropometry	Maternal BMI (kg/m ²)	N=342	36.17 (4.98)	N=356	36.31 (4.69)
	Systolic blood pressure (mmHg)	N=340	117.90 (11.15)	N=352	119.32 (11.00)
	Sum of skin folds (mm) [†]	N=337	124.34 (28.46)	N=354	122.18 (25.06)
Maternal Antenatal and postpartum history					
Gestational diabetes**		N=336	97 (28.9)	N=346	93 (26.9)
Pre-eclampsia [∞]		N=340	11 (3.2)	N=353	11 (3.1)
Total gestational weight gain from pre-pregnancy weight [¶]		N=320	6.92 (4.65)	N=332	7.83 (4.41)
Maternal 6 month postpartum BMI (kg/m ²)		N=345	36.26 (5.14)	N=355	36.45 (5.41)
Change in maternal weight from 15-18 weeks to 6 months postpartum (kg)		N=344	-0.37 (7.41)	N=355	0.36 (6.71)
Infant demographics					
Infant age at 6 months follow up (months)		N=342	5.80 (0.65)	N=356	5.85 (0.72)
Gestation at birth (weeks)		N=342	39.73 (1.54)	N=356	39.55 (2.29)
Birthweight (grams)		N=342	3479.23 (529.40)	N=356	3436.55 (604.09)
Large for Gestational Age >90 th (customised) [‡]		N=342	30 (8.8)	N=356	27 (7.6)
Neonatal feeding history at 72 hrs	Artificial feeding	N=341	63 (18.5)	N=354	78 (22.0)
	Breast feeding	N=341	213 (62.5)	N=354	216 (61.0)
	Partially breastfeeding	N=341	65 (19.1)	N=354	60 (17.0)

Table 5.3: Maternal and infant demographics by randomisation allocation at 6 months' postpartum visit (n=698).

[^] Maternal current smoking at 15-18 weeks' gestation significantly different between intervention and control groups ($p=0.02$). ^{*}IMD quintiles are calculated for the region of residence, by fifths of the population. UK wide-scores were developed by reconciling Scottish data to English norms. ^{**} Gestational diabetes diagnosis by International Association of Diabetes in Pregnancy Study Group criteria at 27⁺⁰ to 28⁺⁶ weeks' gestation. [†] Sum of skinfold thicknesses calculated by the addition of biceps, triceps, suprailiac and subscapular skinfold measurements each measured in triplicate. [∞] Pre-eclampsia defined as systolic blood pressure ≥ 140 mm Hg.

diastolic blood pressure ≥ 90 mm Hg, or both, on at least two occasions 4 hours apart, with proteinuria ≥ 300 mg/24 hours. ¶ Gestational weight gain calculated using estimated weight before pregnancy per the Institute of Medicine Weight Management in Pregnancy Guidelines. † Customised birthweight centile calculated adjusting for maternal height and weight, ethnic origin, parity and sex of the infant.

	Intervention		Control		Mean Diff/ Risk Ratio* (95% CI)	p-value
		Mean (SD)		Mean (SD)		
Subscapular skinfold thickness z-scores**	N=267	0.08 (1.37)	N=280	0.36 (1.37)	-0.26 (-0.49 to -0.02)	0.03
Subscapular skinfold thickness (mm)	N=267	7.55 (1.86)	N=281	7.95 (2.03)	-0.38 (-0.70 to -0.06)	0.02
Triceps skinfold thickness z-scores**	N=296	0.10 (1.56)	N=298	0.24 (1.43)	-0.14 (-0.38 to 0.10)	0.25
Triceps skinfold thickness (mm)	N=307	9.69 (2.76)	N=320	9.87 (2.69)	-0.22 (-0.64 to 0.20)	0.31
Sum of skinfolds (mm)	N=267	17.08 (3.93)	N=280	17.71 (3.97)	-0.63 (-1.30 to 0.04)	0.06
BMI for age z-scores**	N=317	-0.07 (1.86)	N=320	0.04 (1.78)	-0.12 (-0.40 to 0.16)	0.39
Abdominal circumference (cm)	N=329	43.74 (4.73)	N=347	43.72 (6.27)	0.07 (-0.78 to 0.92)	0.87
Total body fat estimation (%) †	N=267	19.40 (5.00)	N=280	20.20 (5.07)	-0.80 (-1.65 to 0.04)	0.06
Weight (kg)	N=332	7.93 (1.07)	N=345	8.03 (1.08)	-0.09 (-0.24 to 0.06)	0.25
Weight for age z-scores**	N=321	0.20 (1.08)	N=322	0.29 (1.12)	-0.09 (-0.26 to 0.08)	0.29
Length (cm)	N=321	67.41 (8.58)	N=338	66.37 (12.37)	1.21 (-0.40 to 2.81)	0.14
Length z-scores**	N=309	0.53 (1.79)	N=313	0.55 (1.89)	-0.02 (-0.30 to 0.27)	0.92
Weight for length z-score **	N=314	-0.08 (1.79)	N=324	0.08 (1.63)	-0.18 (-0.45 to 0.09)	0.18
Mid Upper Arm circumference (cm)	N=329	15.30 (1.49)	N=347	15.39 (2.08)	-0.10 (-0.39 to 0.19)	0.51
Crown rump length (cm)	N=186	45.16 (2.89)	N=195	45.14 (3.06)	0.04 (-0.57 to 0.65)	0.90
Occipitofrontal circumference (cm)	N=327	43.69 (3.48)	N=343	43.81 (4.21)	-0.10 (-0.69 to 0.49)	0.75

Table 5.4: Infant anthropometry by UPBEAT randomisation allocation at 6 months' postpartum visit (n=698).

*Treatment effect adjusted for minimisation variables of randomisation (maternal BMI, ethnicity and parity), infant age at 6 month follow up and infant sex. **Z-scores calculated using WHO Anthro; version 3.2.2. † Total body fat estimation calculated using sex-specific equations utilising 2 infant skinfold thicknesses (Slaughter et al., 1988).

	Intervention		Control		Mean Diff/ Risk Ratio* (95% CI)	p-value
		Mean (SD)/N (%)		Mean (SD)/N (%)		
Subscapular (mm)	N=267	7.55 (1.86)	N=281	7.95 (2.03)	-0.39 (-0.71 to -0.07)	0.02
Triceps (mm)	N=307	9.69 (2.76)	N=320	9.87 (2.69)	-0.21 (-0.64 to 0.21)	0.33
Sum of skinfolds (mm)	N=267	17.08 (3.93)	N=280	17.71 (3.97)	-0.63 (-1.30 to 0.03)	0.06
Total body fat estimation (%) ‡	N=267	19.40 (5.00)	N=280	20.20 (5.07)	-0.81 (-1.66 to 0.03)	0.06
Weight (kg)	N=332	7.93 (1.07)	N=345	8.03 (1.08)	-0.10 (-0.25 to 0.06)	0.22
Height (cm)	N=321	67.41 (8.58)	N=338	66.37 (12.37)	1.31 (-0.33 to 2.96)	0.12
Weight z-scores**	N=321	0.20 (1.08)	N=322	0.28 (1.13)	-0.10 (-0.27 TO 0.08)	0.25
BMI z-scores**	N=321	-0.09 (1.87)	N=313	0.01 (1.78)	-0.14 (-0.42 to 0.15)	0.35
Weight for length z-scores**	N=314	-0.08 (1.79)	N=324	0.08 (1.63)	-0.20 (-0.46 to 0.07)	0.15
Abdominal circumference (cm)	N=329	43.74 (4.73)	N=347	43.72 (6.27)	0.01 (-0.82 to 0.85)	0.98
Arm circumference (cm)	N=329	15.30 (1.49)	N=347	15.39 (2.08)	-0.10 (-0.39 to 0.19)	0.50
Crown length (cm)	N=186	45.16 (2.89)	N=195	45.14 (3.06)	0.04 (-0.57 to 0.65)	0.91
Occipitofrontal circumference (cm)	N=327	43.69 (3.48)	N=343	43.81 (4.21)	-0.14 (-0.70 to 0.41)	0.61

Table 5.5: Sensitivity analysis for infant outcomes adjusting for maternal smoking to determine the influence of the UPBEAT intervention on infant anthropometry at 6 month of age (n=698).

*Treatment effect adjusted for minimisation variables of randomisation (maternal BMI, ethnicity and parity), infant age at 6 month follow up and infant sex and maternal smoking status at 15-18⁺⁶ weeks' gestation **Z-scores calculated using WHO Anthro; version 3.2.2 (de Onis and Blössner, 2003) .‡ Infant total body fat estimation calculated using sex-specific equations with two skin fold measurements as published by Slaughter et al (1988)(Slaughter et al., 1988).

	Observations	Missing data	% missing data	Effect size using MI*	P-value
Subscapular skinfold thickness (mm)	548	972	63.95	-0.29(-0.63 to -0.06)	0.04
Triceps skinfold thickness (mm)	627	893	58.75	-0.10 (-0.52 to 0.32)	0.61
Sum of skinfolds thickness (mm)	547	973	64.01	-0.63 (-1.31 to 0.06)	0.06
Total body fat estimation (%)	547	973	64.01	-0.79 (-1.66 to 0.72)	0.07
Weight (kg)	677	843	55.46	-0.08 (-0.20 to 0.04)	0.18
Height (cm)	659	861	56.64	0.83 (-0.45 to 2.12)	0.19
Weight z-scores	643	877	57.70	-0.09 (-0.27 to 0.09)	0.31
Length z-scores	622	898	59.08	-0.16 (-0.45 to 0.42)	0.93
BMI z-scores	622	898	59.08	-0.11 (-0.49 to 0.28)	0.55
Weight for length z-scores	638	882	58.03	-0.13 (-0.46 to 0.20)	0.40
Abdominal circumference (cm)	676	844	55.53	-0.01 (-0.87 to 0.85)	0.98
Mid-arm circumference (cm)	676	844	55.53	-0.01 (-0.41 to 0.39)	0.95
Crown-rump length (cm)	381	1139	74.93	0.08 (-0.92 to 1.09)	0.84
Occipitofrontal circumference (cm)	670	850	55.92	-0.02 (-0.52 to 0.49)	0.95

Table 5.6: Multiple imputation to increase precision of treatment estimates of the effect of the UPBEAT intervention on infant anthropometry at 6 months.

*Multiple imputation methodology; Data was imputed to create 50 datasets using 10 burn-in iterations for live-born infants using maternal trial entry BMI, age, ethnicity, parity, early pregnancy smoking status, randomisation allocation, measures of maternal anthropometry including GWG, maternal diet at 27-28⁺⁶ weeks' and 6 months postpartum (glycaemic load, glycaemic index, saturated fat, carbohydrate, protein, energy intake), maternal physical activity at 27-28⁺⁶ weeks' and 6 months post-partum (MET), gestation at delivery, infant sex, age at follow-up, mode and duration of early feeding, sleep, child health, duration of hospital admissions. Analyses were performed by intention to treat. *Treatment effects were estimated on pooled datasets using risk ratio (binary outcomes) and mean difference (continuous outcomes) adjusted for minimisation variables (Maternal BMI, parity and ethnicity) infant sex and age at follow-up visit. Abbreviations: MI- Multiple imputation*

δ	Difference	Standard error
0.5	-0.38	0.16
0.75	-0.38	0.16
1	-0.37	0.16
1.5	-0.36	0.17
2	-0.35	0.17

Table 5.7: Sensitivity analysis for departure from missing at random assumption for the effect of the UPBEAT intervention on infant subscapular skinfold thickness at 6 months of age.

δ	Difference	Standard error
0.5	-0.16	0.21
0.75	-0.15	0.22
1	-0.15	0.22
1.5	-0.14	0.22
2	-0.13	0.23

Table 5.8: Sensitivity analysis for departure from missing at random assumption for the effect of the UPBEAT intervention on infant triceps skinfold thickness at 6 months of age.

	Intervention		Control		Diff/ Risk ratio*	p-value
	Mean (SD)/N (%)		Mean (SD)/N (%)			
Infant feeding at 6 months						
Breast milk only	N=336	9 (2.7)	N=347	10 (2.9)	1.01 (0.42 to 2.43)	0.98
Mixed feeding	N=336	55 (16.4)	N=347	62 (17.9)	0.97 (0.70 to 1.33)	0.83
Breast milk & solids	N=336	57 (17.0)	N=347	61 (17.6)	0.98 (0.71 to 1.36)	0.92
Formula & solids	N=336	193 (57.4)	N=347	193 (55.6)	1.05 (0.93 to 1.19)	0.40
Formula only	N=336	22 (6.5)	N=347	21 (6.1)	1.09 (0.62 to 1.95)	0.76
Days exclusively breast fed	N=260	80.57 (65.11)	N=243	85.04 (65.60)	-4.81 (-16.18 to 6.57)	0.41
Appetite and satiety **						
Enjoyment of food	N=293	18.54 (2.23)	N=314	18.40 (2.45)	0.15 (-0.22 to 0.52)	0.43
Food responsiveness	N=342	11.94 (4.95)	N=350	12.34 (4.87)	-0.39 (-1.12 to 0.33)	0.29
General appetite	N=342	3.56 (1.31)	N=350	3.65 (1.18)	-0.08 (-0.27 to 0.10)	0.38
Slowness in eating	N=342	10.08 (2.62)	N=350	10.26 (2.55)	-0.18 (-0.56 to 0.20)	0.35
Satiety responsiveness	N=341	6.49 (2.63)	N=350	6.46 (2.48)	0.02 (-0.36 to 0.40)	0.91
Childcare						
Childcare centre	N=260	19 (7.3)	N=274	18 (6.6)	1.05 (0.56 to 1.95)	0.88
Family Member	N=260	237 (91.2)	N=274	244 (89.1)	1.03 (0.98 to 1.09)	0.29
Nanny	N=260	5 (1.9)	N=274	6 (2.2)	0.90 (0.27 to 2.94)	0.86
Other	N=260	4 (1.5)	N=274	9 (3.3)	0.44 (0.14 to 1.42)	0.17
Sleep						
Total infant sleep (hours)	N=340	14.57 (11.17)	N=353	13.54 (9.68)	1.04 (-0.52 to 2.60)	0.19
Child health						
Total inpatient nights	N=35	3.97 (3.63)	N=33	2.67 (2.07)	2.03 (0.25 to 3.81)	0.03

Table 5.9: Postnatal characteristics previously implicated with infant adiposity, by UPBEAT randomisation allocation at 6 months follow-up visit (n=698).

*Treatment effect adjusted for minimisation variables of randomisation (maternal BMI, ethnicity and parity), infant age at 6 month follow up and infant sex. ** Appetite and satiety assessed by the Baby Eating Behaviour Questionnaire (Llewellyn et al., 2011).*

	Intervention		Control		Risk ratio*	P-value
	N (%)		N (%)			
Reason for hospital admission						
Gastrointestinal	N=35	2(5.7)	N=33	9 (27.3)	0.21 (0.05 to 0.92)	0.04
Viral or bacterial infection	N=35	15 (42.9)	N=33	10 (30.3)	1.44 (0.77 to 2.71)	0.26
Weight loss	N=35	3 (8.6)	N=33	2 (6.1)	1.47 (0.26 to 8.23)	0.66
Congenital anomaly	N=35	3 (8.6)	N=33	2 (6.1)	1.51 (0.27 to 8.53)	0.64
Respiratory	N=35	7 (20.0)	N=33	4 (12.1)	1.87 (0.61 to 5.73)	0.27
Hypoglycaemia	N=35	0 (0.0)	N=33	1 (3.0)	-	-
Other	N=35	5 (14.3)	N=33	5 (15.2)	-	1.00
Reasons for use of medications						
Infection	N=491	96 (19.6))	N=488	76 (15.6)	1.20 (0.91 to 1.57)	0.19
Other	N=491	147 (29.9)	N=488	154 (31.6)	0.96 (0.80 to 1.16)	0.69
Pain	N=491	174 (35.4)	N=488	182 (37.3)	0.96 (0.82 to 1.14)	0.67
Pyrexia	N=491	74 (15.1)	N=488	76 (15.6)	0.96 (0.71 to 1.28)	0.76

Table 5.10: Cause of infant hospital admissions and medication use by randomisation allocation in offspring born to obese women.

* Difference/ risk ratio adjusted for maternal pre-pregnancy BMI, parity, ethnicity and infant sex and age at follow up.

Infant	≥4 HTS		≤3 HTS		Mean diff/ Risk Ratio*	p-value
Subscapular (mm)	N=83	7.74 (1.66)	N=184	7.46 (1.94)	0.09 (-0.40 to 0.58)	0.72
Triceps (mm)	N=98	9.12 (2.63)	N=209	9.95 (2.78)	-0.64 (-1.31 to 0.03)	0.06
Sum of skinfolds (mm)	N=83	16.94 (3.92)	N=184	17.15 (3.94)	-0.22 (-1.32 to 0.89)	0.70
Total body fat estimation (%)‡	N=83	19.35 (5.08)	N=184	19.42 (4.97)	-0.24 (-1.66 to 1.17)	0.73
Infant weight (kg)	N=103	7.71 (1.01)	N=229	8.02 (1.09)	-0.21 (-0.45 to 0.03)	0.08
Weight z-scores**	N=102	0.07 (1.08)	N=219	0.25 (1.07)	-0.20 (-0.46 to 0.06)	0.13
Abdominal circumference (cm)	N=103	42.66 (6.73)	N=226	44.23 (3.37)	-1.53 (-3.17 to 0.12)	0.07
Arm circumference (cm)	N=103	15.00 (1.86)	N=226	15.44 (1.27)	-0.44 (-0.91 to 0.02)	0.06

Table 5.11: Infant anthropometry according to number of maternal health trainer sessions within the intervention arm of the UPBEAT randomised controlled trial (n=342).

*Difference/ risk ration adjusted for maternal pre-pregnancy BMI, parity, ethnicity and infant sex and age at follow up. ‡ Infant total body fat estimation calculated using sex-specific equations with two skin fold measurements as published by Slaughter et al (Slaughter et al., 1988). **Z-scores calculated using WHO Anthro; version 3.2.2 (de Onis and Blössner, 2003)

	Female		Male		Wald test for interaction
	Diff/ Risk Ratio*	p-value	Diff/Risk Ratio*	p-value	
Subscapular (mm)	-0.50 (-0.97 to -0.02)	0.04	-0.23 (-0.65 to 0.20)	0.29	0.42
Triceps (mm)	-0.19 (-0.80 to 0.42)	0.53	-0.26 (-0.86 to 0.34)	0.39	0.87
Sum of skinfolds (mm)	-0.83 (-1.83 to 0.17)	0.10	-0.41 (-1.30 to 0.48)	0.37	0.55
Total body fat estimation (%) ‡	-1.09 (-2.41 to 0.22)	0.10	-0.49 (-1.56 to 0.58)	0.37	0.14
Infant weight (kg)	-0.13 (-0.34 to 0.08)	0.22	-0.07 (-0.30 to 0.16)	0.55	0.72
Weight z-scores**	-0.14 (-0.36 to 0.08)	0.23	-0.06 (-0.32 to 0.20)	0.65	0.63
Arm circumference (cm)	-0.06 (-0.52 to 0.40)	0.80	-0.11 (-0.43 to 0.21)	0.51	0.81
Abdominal circumference (cm)	0.30 (-0.89 to 1.49)	0.62	-0.33 (-1.48 to 0.82)	0.58	0.35

Table 5.12: Pre-defined interaction tests for infant anthropometry by offspring sex stratified by UPBEAT randomisation allocation (n=698).

**Treatment effect adjusted maternal pre-pregnancy BMI, parity, ethnicity and infant sex and age at follow up. Differences and risk ratios are calculated by randomisation allocation. ** Z-scores calculated using WHO Anthro; version 3.2.2 (de Onis and Blössner, 2003). ‡ Infant total body fat estimation calculated using sex-specific equations with two skin fold measurements as published by Slaughter et al (Slaughter et al., 1988).*

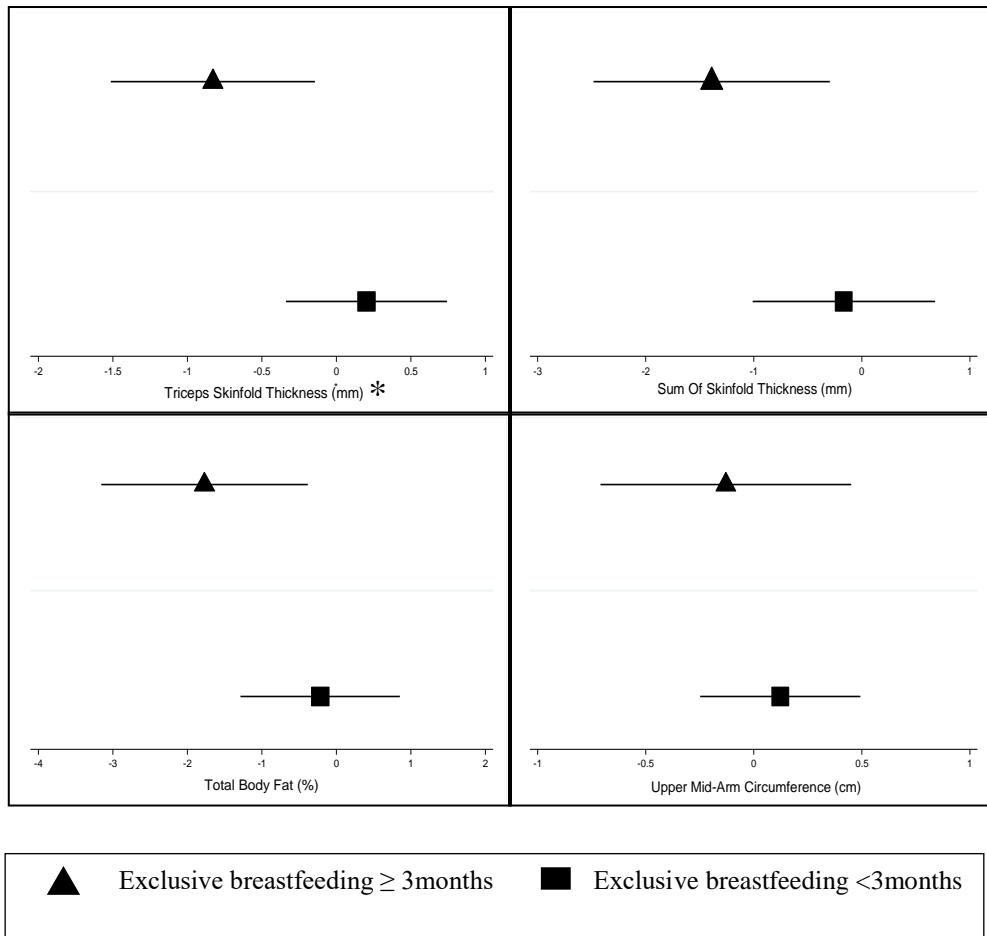


Figure 5.2: Relationship between duration of exclusive breastfeeding and anthropometry measured at 6 months postpartum in infants from the UPBEAT trial (n=698).

Effect estimates/ mean differences plotted with 95% confidence intervals. For triceps skinfold thickness (n=627), sum of skinfold thickness (n=547), total body fat (n=547) and upper mid-arm circumference (n=676).

**Significant Wald test for interaction $p < 0.05$*

	<3months breastfeeding		≥ 3 months breastfeeding		Wald test for interaction
	Diff/ Risk Ratio*	p-value	Diff/ Risk Ratio*	p-value	
Subscapular (mm)	-0.27 (-0.66 to 0.12)	0.18	-0.54 (-1.10 to 0.02)	0.06	0.44
Triceps (mm)	0.148 (-0.36 to 0.71)	0.52	-0.90 (-1.59 to -0.21)	0.01	0.02
Sum of skinfolds (mm)	-0.18 (-1.02 to 0.67)	0.68	-1.42 (-2.52 to -0.32)	0.01	0.09
Total body fat estimation (%) ‡	-0.23 (-1.30 to 0.85)	0.68	-1.81 (-3.20 to -0.42)	0.01	0.09
Infant weight (kg)	-0.05 (-0.24 to 0.14)	0.58	-0.16 (-0.42 to 0.10)	0.24	0.58
Weight z-scores**	-0.04 (-0.24 to 0.17)	0.73	-0.18 (-0.48 to 0.11)	0.23	0.49
Abdominal circumference (cm)	0.05 (-0.85 to 0.96)	0.91	0.12 (-1.51 to 1.75)	0.89	0.97
Arm circumference (cm)	0.11 (-0.24 to 0.45)	0.54	-0.36 (-0.74 to 0.03)	0.07	0.08

Table 5.13: Pre-defined interaction tests by infant breastfeeding (defined as >3 months or <3 months) stratified by randomisation to the UPBEAT intervention (n=698).

**Treatment effect adjusted for maternal pre-pregnancy BMI, parity, ethnicity and infant sex and age at follow up. Presented differences and risk ratio are calculated by treatment allocation. ** Z-scores calculated using WHO Anthro; version 3.2.2 (de Onis and Blössner, 2003). ‡ Infant total body fat estimation calculated using sex-specific equations with two skin fold measurements as published by Slaughter et al (Slaughter et al., 1988).*

	Intervention		Standard care		Treatment effect*	P-value
	Mean (SD)/ N (%)		Mean (SD)/ N (%)		Mean Difference (95% CI)	
Maternal diet**						
Glycaemic Load per day	N=116	98.94 (32.80)	N=126	134.69 (62.68)	-35.34 (-48.00 to -22.67)	<0.001
Saturated fat (%E)	N=116	11.89 (2.61)	N=126	13.75 (2.85)	-1.93 (-2.64 to -1.22)	<0.001
Total energy (kcal/day)	N=116	1473.84 (596.60)	N=126	1831.21 (727.65)	-354.52 (-505.95 to -203.10)	<0.001
Glycaemic Index (0-100)	N=116	53.06 (4.06)	N=126	57.04 (3.74)	-3.94 (-4.93 to -2.94)	<0.001
Carbohydrate (%E)	N=116	47.69 (6.71)	N=126	48.03 (6.22)	-0.18 (-1.84 to 1.49)	0.84
Total fat (%E)	N=116	29.70 (4.94)	N=126	32.26 (4.75)	-2.65 (-3.91 to -1.38)	<0.001
Protein (%E)	N=116	22.57 (4.42)	N=126	19.82 (3.94)	2.70 (1.63 to 3.77)	<0.001
Fibre (g/day)	N=116	12.12 (4.36)	N=126	12.27 (6.81)	-0.12 (-1.57 to 1.33)	0.87
Maternal physical activity^		Median (IQR)		Median (IQR)	Median regression (95% CI)	
MET (min/week) †	N=349	2190 (1053, 4158)	N=358	2012 (990, 4088)	93.95 (-264.81 to 452.72)	0.61
MVPA (min/week)	N=349	120 (0, 360)	N=358	120 (0, 360)	10.43 (-39.31 to 60.18)	0.68
Walking (min/week)	N=349	420 (180, 840)	N=358	420 (180, 630)	0.00 (-68.88 to 68.88)	1.00

Table 5.14: Maternal dietary and physical activity data by randomisation allocation at 6 months postpartum (n=698).

Abbreviations: CI- Confidence Intervals; %E- %Energy; g/day- grams per day; kcal/day- kilocalories per day; MET- Metabolic equivalent of task; MVPA- Moderate and Vigorous physical activity. *Treatment effect adjusted for maternal trial entry BMI, parity and ethnicity. ** Maternal diet- Women with a reported energy ≤ 4.5 Mj/day or ≥ 20 Mj/day at 15⁺⁰ -18⁺⁶ weeks' gestation were excluded from the analyses of diet. Dietary intervention estimates were calculated using multiple regression and adjusted for minimisation variables. ^ Physical activity estimates were calculated using bootstrapped (1000 replications), median regression adjusting for minimisation variables. † MET is defined as the energy expenditure ratio of activity to rest; 1 MET is approximately equal to an individual's resting energy expenditure.

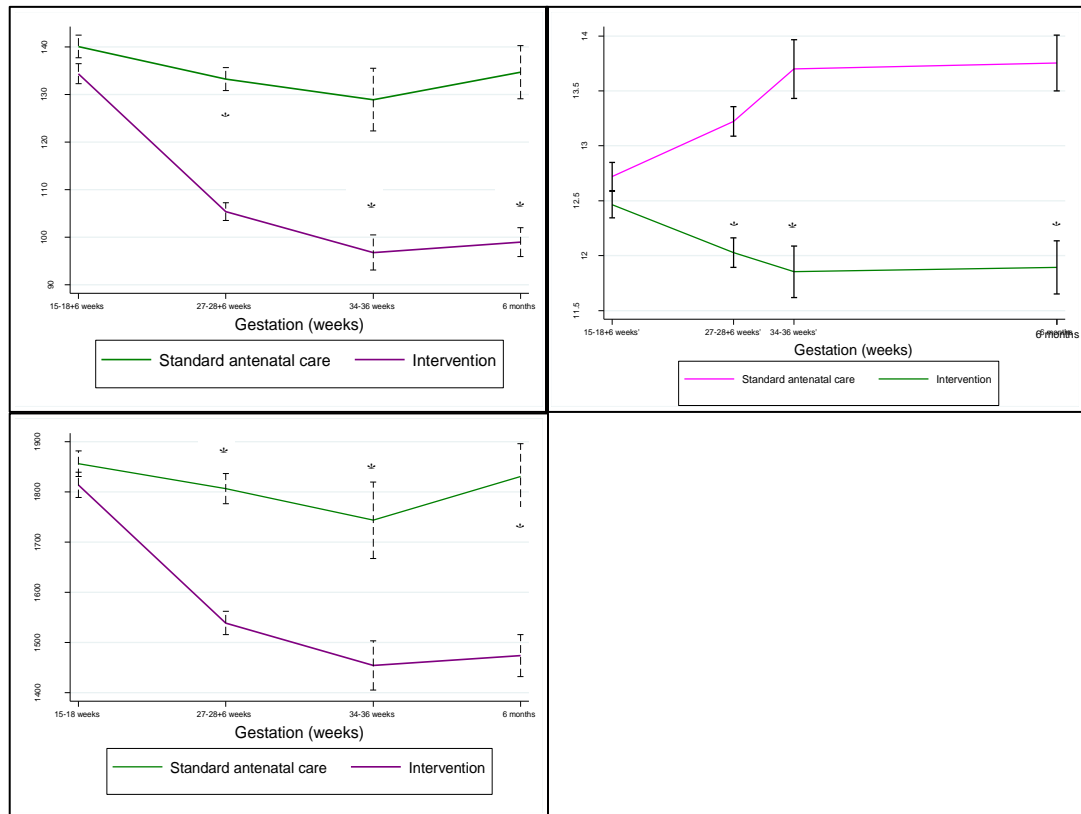
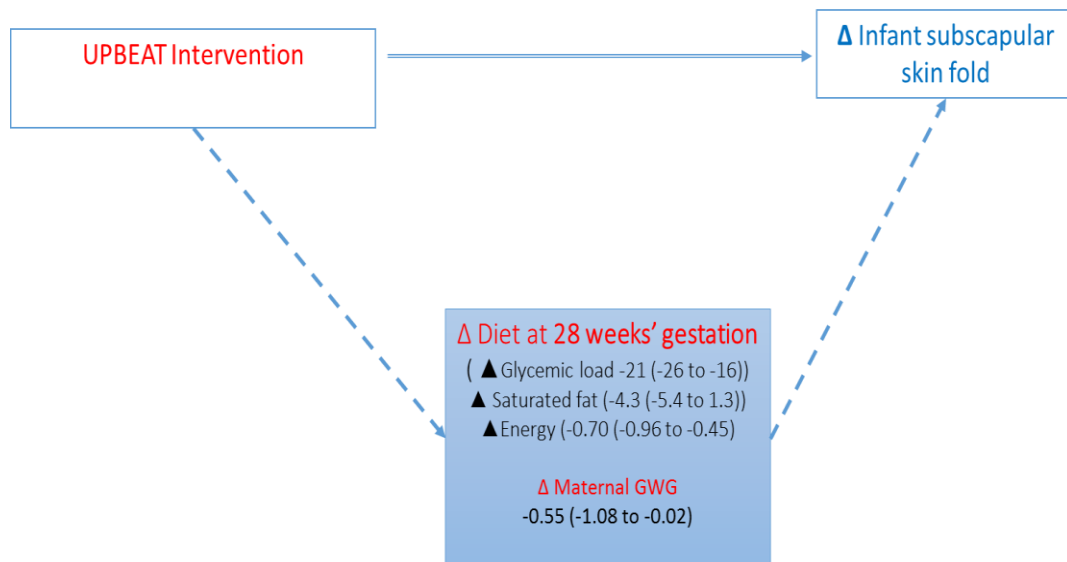


Figure 5.3: Maternal glycaemic load, saturated fat and energy intake at 6 months postpartum by randomisation allocation in obese women enrolled in the UPBEAT randomised controlled trial (n=242).

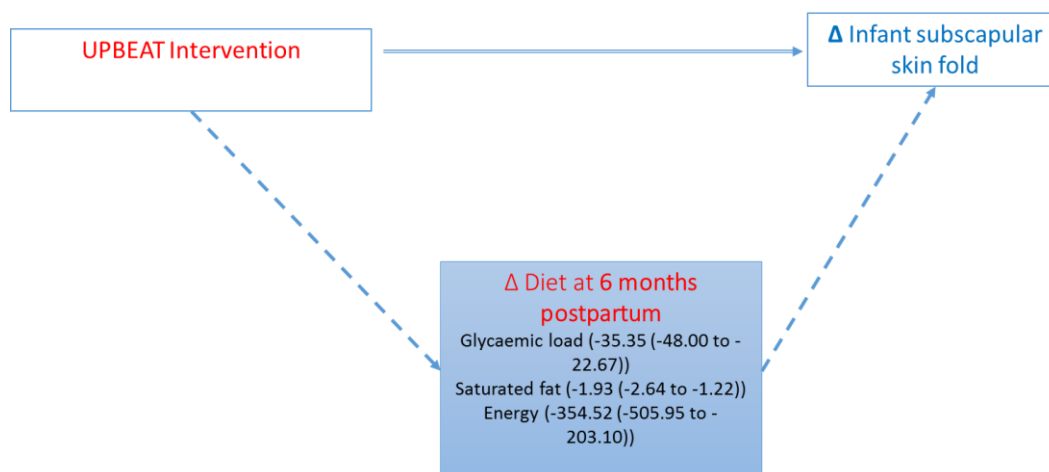
*Abbreviations: %E- Percentage energy; kcal/day- kilocalorie per day. Arithmetic mean with standard error plotted at each gestation (weeks), showing nutritional consumption per day. * $p < 0.05$*



	b	95% CI	p-value
Maternal anthropometry			
Early GWG*	-0.41	-0.73 to -0.09	0.02
Late GWG* *	-0.45	-0.78 to -0.12	0.01
Total GWG	-0.44	-0.78 to -0.11	0.01
Maternal diet			
Glycaemic load at 27-28 ⁺⁶ weeks'	-0.31	-0.65 to 0.04	0.08
Saturated fat at 27-28 ⁺⁶ weeks' (%E)	-0.38	-0.72 to -0.04	0.03
Energy intake at 27-28 ⁺⁶ weeks' (kcal)	-0.34	-0.68 to -0.01	0.05

Figure 5.4: Casual mediation analysis assessing the influence of change in maternal dietary intake and gestational weight gain at 27-28 weeks' mediated on the observed difference in infant subscapular skinfold thickness in offspring born to obese pregnant women.

* Early GWG defined as maternal weight objectively measured at 27-28⁺⁶ weeks' gestation subtracted from weight at trial entry (15-18⁺⁶ weeks' gestation). **Late GWG defined as maternal weight objectively measured at 34-36 weeks' gestation subtracted from maternal weight at 27-28⁺⁶ weeks'. Casual mediation analysis was performed for late gestational weight gain, independent of early GWG. Abbreviations; GL- Glycaemic load, GWG- Gestational weight gain, MET-Metabolic equivalent of task



	Effect	b	95% CI	p-value
Maternal Glycaemic load at 6 months *				
	Direct effect	-0.59	-1.29 to 0.11	0.100
	Indirect effect	0.00	-2.80 to 0.00	0.570
	Total effect	-0.59	-1.29 to 0.11	0.100
Maternal Saturated fat at 6 months *				
	Direct effect	-0.42	-1.11 to 0.27	0.230
	Indirect effect	-	-	-
	Total effect	-0.42	-1.11 to 0.27	0.230
Maternal energy intake at 6 months *				
	Direct effect	-0.62	-1.30 to 0.05	0.076
	Indirect effect	0.015	-0.005 to 0.007	0.623
	Total effect	-0.62	-1.30 to 0.06	0.076

Figure 5.5: Casual mediation analysis assessing the effect of change in maternal diet at 6 months postpartum mediated on infant subscapular skinfold thickness in offspring born to obese pregnant women.

**Casual mediation analyses undertaken for maternal 6-month postpartum dietary intake independent of antenatal (27-28⁺⁶ weeks') dietary intake.*

5.4 Discussion

5.4.1 Overall findings

This study has addressed the effect of a pregnancy lifestyle behavioural intervention in obese women on offspring adiposity, maternal diet and physical activity at 6 months postpartum. This study, for the first time has found that a dietary and physical activity intervention in pregnant women with obesity was associated with a reduction in a measure of offspring adiposity, and that changes in maternal diet during pregnancy persisted into the postnatal period. Further analyses suggested that the effect of the intervention on offspring adiposity was independently mediated by the observed reduction in maternal gestational weight gain, dietary fat and energy intake in pregnancy and therefore an expectation that lifestyle interventions have the potential to reduce offspring adiposity. Subscapular skinfold thickness, in comparison to the other anthropometric measurements assessed, is recognised as an accurate index of central adiposity, with a generally lower measurement error than triceps skinfold thickness (Godfrey *et al.*, 1996b; Silventoinen *et al.*, 2009). In children and adults, subscapular skinfold thickness has been related to impaired glucose metabolism, and in adolescents to increased serum cholesterol concentrations (Srinivasan *et al.*, 2003; Santos *et al.*, 2016). It is plausible, therefore that the maternal dietary and weight changes resulting from the intervention may influence infant body composition towards a healthier metabolic profile (Srinivasan *et al.*, 2003; Craig *et al.*, 2008; Renault *et al.*, 2015).

5.4.2 The effect of the UPBEAT intervention on infant adiposity at 6 months of age

Although the magnitude of difference in this measure of adiposity (subscapular skinfold thickness) between intervention and controls arms was modest (5%), it reflected a 0.26 reduction in z-score, which incorporated adjustment for infant sex and age. Indications from mother-child cohorts, including the USA Project Viva study, suggest that even modest differences in body composition at age 6 months may be amplified as the child grows older, and that this may be apparent as early as 3 years (Taveras *et al.*, 2009). The

Bogalusa Heart Study observed that greater offspring childhood subscapular skinfold thickness related to parental type 2 diabetes was associated with a subsequent adverse metabolic profile in early adulthood (Srinivasan *et al.*, 2003). Any persistent influence of the intervention on childhood obesity will only be revealed as the children are older, but an abundance of evidence suggests that increased adiposity tracks from infancy, through childhood to adulthood (Cunningham *et al.*, 2014).

We are aware of only two relevant similar studies. The first, the Lifestyle in Pregnancy study (LIP) (Vinter *et al.*, 2011), assessed body composition in older infants (2.8 years) of obese mothers (n=157) who had been randomised to an antenatal lifestyle intervention with the primary aim of reducing gestational weight gain. No change in infant total fat mass, as assessed by dual energy x-ray absorptiometry scan, was observed (Tanvig *et al.*, 2014). However, it was not reported whether this intervention modified specific components of maternal antenatal diet or body composition, although a reduction in median gestational weight gain was observed. Secondly, a recent randomised controlled trial of a low glycaemic diet, but in women of heterogeneous BMI, found no difference in infant body composition at 6 months of age between intervention and control arms, despite a reduction in neonatal thigh circumference (Donnelly *et al.*, 2015; Horan *et al.*, 2016). The difference between these studies and UPBEAT may relate to the greater intensity of the UPBEAT intervention, involving 8 contact sessions with health trainers, at weekly intervals (Poston *et al.*, 2015).

5.4.3 *Maternal postpartum diet composition and physical activity*

There remains a paucity of data regarding the long-term efficacy of lifestyle interventions in obese pregnant women (Patel *et al.*, 2015). This study has shown that dietary advice focussing on a reduction of maternal insulin resistance, as a component of a complex intervention, can have a prolonged effect which may have potential to improve long term

health as well as familial nutritional environment (Owen *et al.*, 2005; Robinson *et al.*, 2007; Ranjit *et al.*, 2015). Although this study found significant differences in maternal nutritional composition, no differences were observed between groups in maternal BMI or measures of adiposity at 6 months postpartum. A sustained effect of any maternal dietary intervention on maternal dietary intake postpartum has to our knowledge not been reported previously. In contrast, in the LIMIT trial, follow up of 50.5% of participants, reported no difference in maternal dietary composition at 4 months postpartum, also by self-report (Dodd *et al.*, 2014a). The lower magnitude of intervention effects on maternal dietary variables compared with UPBEAT may explain these differences.

Using the method of causal mediation analysis, this study found evidence that the lower dietary saturated fat and energy intake at 28 weeks' gestation induced by the UPBEAT intervention, rather than the change in glycaemic load, was associated with the reduction in infant subscapular skinfold thickness at 6 months of age. The reduction in gestational weight gain irrespective of timing and total gestational weight gain were also directly associated with the observed difference. These observations would concur with several reports describing associations between maternal gestational weight gain or diet and offspring adiposity (Oken *et al.*, 2007; Okubo *et al.*, 2014). Antenatal interventions shown to improve maternal diet and subsequently reduce gestational weight gain may therefore be pragmatic and effective measures to reduce early infant adiposity.

5.4.4 *Maternal breastfeeding and infant adiposity*

The observation that exclusive breastfeeding for more than 3 months may interact with the maternal intervention to reduce offspring triceps skinfold thickness provides some evidence that breast feeding may compound the benefits of the maternal intervention, although caution should be exercised in over-interpretation as the study was not powered to test interactions such as these. The role of other intrauterine exposures remains to be

elucidated; whilst we previously reported no differences in fasting lipids, C-peptide and insulin at 28 weeks' gestation between randomisation arms (Poston *et al.*, 2015), ongoing biochemical and metabolomic analyses in maternal and cord blood may provide insight into mechanistic pathways.

5.4.5 *Strengths and Limitations*

A limitation of this study was the follow up of only 47.3% of those infants eligible from the original randomised controlled trial (Poston *et al.*, 2015), but this was similar to the rate of follow up of recently published randomised controlled trials in pregnant women (Tanvig *et al.*, 2014; Landon *et al.*, 2015; Horan *et al.*, 2016). Due to the stringent inclusion of only complete dietary questionnaires, maternal dietary data was calculated only for 34.2% of the mothers. The dietary data was collected by self-report but compared favourably to a more rigorous method (triple pass 24hr recall) as assessed in the pilot trial (Poston *et al.*, 2013). Strengths of the study include the prospective collection of in-depth data addressing familial and individual determinants of infant adiposity, and of maternal *in-utero* exposures. The richness of data in the UPBEAT study can be considered both a strength and limitation. Whilst providing comprehensive information relevant to developmental origins of early infant obesity, and assessment of mediation effects, limits are imposed on interpretation of secondary analyses in the context of multiple testing.

5.4.6 *Conclusion*

In conclusion, this study provides evidence of the potential for targeted intervention in obese women to improve health for the mother and her offspring. Pregnancy, as demonstrated in this study, appears to be a pragmatic 'teachable' moment for initiating long-term healthier dietary behaviours in the mother and reducing a physiologically relevant measure of adiposity in the offspring.

Chapter 6 Role of early feeding on infant anthropometry

6.1 Introduction

The development of childhood obesity is multifactorial. A combination of exposure to an adverse *in-utero* environment as well as sub-optimal postnatal feeding and rapid growth trajectories are likely to play a contributory role, as discussed in *Chapter 1* (Patel *et al.*, 2015). This chapter focusses on two facets of early life feeding; firstly, relationships between breast feeding and infant growth and body composition at six months, and secondly, the relationship between infant eating behaviours and anthropometric outcomes in children whose mothers participated in the UPBEAT study.

Rapid infant weight gain within the first year of life has been associated with an increased risk of later obesity and raised systolic blood pressure in mid-childhood (Baird *et al.*, 2005; Druet *et al.*, 2012; Weng *et al.*, 2012; Ong *et al.*, 2015; Perng *et al.*, 2016). Recent evidence supports the notion that the trajectory of growth associated with the development of later obesity is established in early infancy in offspring born to mothers of heterogeneous BMI (Stuart and Panico, 2016). Observational studies have implicated a role of early infancy nutritional status with the development of childhood obesity, thereby providing convincing evidence that early life mode and/or intensity of feeding may be a potential target, amenable to intervention, for the prevention of childhood obesity. One mechanism by which mode of infant feeding could lead to obesity in later life is through modification of early infant growth velocities, differentiation of adipocyte precursors and satiety and appetite regulation (Patel *et al.*, 2015).

Previous population studies have demonstrated that breastfeeding may be protective against later obesity; however these studies have been based on measures of offspring weight (Owen *et al.*, 2005) as opposed to direct measurement of adiposity, and therefore lack the

potential to disentangle the associations of early growth and mode of feeding with different components of body composition. The mechanisms remain speculative but could relate to reduced early growth trajectories or to other biological pathways, for example, persistent modification of the central appetite and satiety mechanisms (Bartok and Ventura, 2009; Berry *et al.*, 2013).

At present, the WHO recommends exclusive breastfeeding for the first 6 months of life with subsequent introduction of solids. Despite the known benefits of breastfeeding, obese women are less likely to breastfeed exclusively and more likely to introduce solids < 4 months in comparison to lean women (Li *et al.*, 2003). Since, specific influences of obese maternal feeding practices in relation to early infancy body composition remain unknown, this study sought to address the relationship between mode of early life feeding, age at weaning on measures of adiposity and growth in infants at 6 months of age.

Few studies have addressed the relationship between offspring feeding behaviours and adiposity in early infancy (Agras *et al.*, 1987; Taveras *et al.*, 2004; Llewellyn *et al.*, 2010). In one of these, utilising the validated Baby Eating Behaviour Questionnaire, 4 obesogenic appetite traits were identified and associated with weight gain from birth to 2 years (Llewellyn *et al.*, 2011; Quah *et al.*, 2015). This was prompted by previous experimental studies in animals suggesting a relationship between maternal obesity and offspring hyperphagia as described in *Chapter 1: Introduction* (Patel *et al.*, 2015); this relationship mediated through fetal leptin known to influence neurodevelopment of the energy control centres of the hypothalamus (Ross and Desai, 2014). However, in human pregnancy cord leptin concentrations have been associated with approximately 10-15% of the variation in offspring weight, suggesting a more important influence of postnatal environmental factors (Ong *et al.*, 1999; Mantzoros *et al.*, 2009; Kaar *et al.*, 2014).

The primary aim of this study was to assess the role of early life feeding practices, including mode of feeding and age at introduction of solids, and measures of body composition and growth in infants at 6 months, born to obese women. Since mechanisms controlling appetite and satiety are considered to develop in early infancy, relationships between mode of feeding and appetite and satiety were also studied. A third aim, was to investigate the relationship between measures of infant appetite and satiety with infant anthropometry at 6 months of age.

6.2 Research design and methods

6.2.1 Study design

The study design was a cohort analyses using complete cases from the UPBEAT dataset.

6.2.2 Study population

Details of the UPBEAT study population including trial inclusion and exclusion criteria are provided in *Chapter 2: Methods*.

Mother and infant pairs were included within this analysis if they had attended the follow-up visit at 6 months of age, completed a feeding and growth questionnaire and if detailed infant anthropometric data were available at 6 months of age. Infants were excluded if suffering from significant ill-health at 6 months, or born ≤ 34 weeks' gestation. As preterm infants (born ≤ 34 weeks' gestation) have significantly different growth trajectories in early infancy, and in appetite and satiety measures compared to infants born at term, they were excluded from the analysis (Llewellyn *et al.*, 2010; Gong *et al.*, 2013).

6.2.3 Outcomes

The primary outcome for this study was measures of infant adiposity as assessed by subscapular and triceps SFT z-scores derived using the WHO reference population (WHO, 2006). Secondary outcomes included weight, length, BMI and mid-upper arm circumference z-scores, SSFT (mm), estimated body fat (%) derived from sex specific equations and infant weight and length gain velocity (Slaughter *et al.*, 1988; WHO, 2006). Further assessment was made for overweight and obesity in early infancy, catch up and catch down growth as per WHO definitions. Associated definitions and methodology for infant anthropometric outcomes are described in detail in *Chapter 2: Methods*.

Infant weight gain velocity in the first 6 months were calculated as;

$$\text{Weight gain velocity (g/month)} = \frac{\text{Infant weight at 6 months} - \text{Birthweight}}{\text{Exact age at 6 month follow-up (months)}}$$

Infant length velocity within the first 6 months were calculated as;

$$\text{Length gain velocity (cm/month)} = \frac{\text{Infant length at 6 months} - \text{Length at birth}}{\text{Exact age at 6 month follow-up (months)}}$$

6.2.4 Exposures

6.2.4.1 Early feeding and growth questionnaire

Data regarding mode and duration of early feeding were collected using a validated feeding and growth questionnaire addressing mode, duration of early feeding and weaning history. Information was obtained on whether infants received anything other than breast milk during this period including water, sugar-sweetened beverages and food and mineral supplements. Questionnaires were administered by trained midwives at the 6 months postpartum study visit. Definitions of early life feeding exposures were derived based on previous studies (Huh *et al.*, 2011), and are summarised in Table 6.1.

Exposure	Definition
Exclusive breastfeeding	Exclusive breastfeeding ≥ 4 months (122 days) of age where infants did not receive anything else with the exception of water
Formula feeding	Last episode of breastfeeding ≤ 2 months (61 days) of age where data was also provided on the age of introduction of formula feeding and type of formula milk provided
Mixed feeding	Last episode of breastfeeding > 2 months and ≤ 4 months of age In scenarios where predominately breastfed infants received supplements of sugar sweetened beverages, this was classed as mixed feeding.

Table 6.1: Definitions of early mode of feeding exposure used in investigating the role of early infant feeding on body composition at 6 months of age.

6.2.4.2 Introduction of solid foods

This was defined as the age of introduction to any type of solid foods. Recent evidence has shown that a large proportion of infants are weaned at four months of age in offspring born

to women of heterogeneous BMI, despite WHO guidance (Chivers *et al.*, 2010; Huh *et al.*, 2011; Barrera *et al.*, 2016). In accordance with previously published data, the average weaning age was categorised as “introduction to solids ≤ 4 months”, “introduction to solids between 4-5 months” and “introduction of solids ≥ 6 months”

6.2.4.3 Infant appetite and satiety questionnaire

The validated Baby Eating Behaviour Questionnaire was used to assess infant appetite and satiety at 6 months of age (Llewellyn *et al.*, 2011). This 18-item questionnaire provides a standardised measure to characterise infant appetite characteristics, previously associated with excessive weight gain in children (Llewellyn *et al.*, 2011). Mothers were required to score their baby’s feeding style during a ‘typical daytime feed’ and responses varied from never (1) to always (5). The questionnaire was adapted from the Children’s Eating Behaviour Questionnaire which identified five distinctive appetite traits; enjoyment of food, food responsiveness, slowness in eating, satiety responsiveness and general appetite (Llewellyn *et al.*, 2011). Questionnaires were analysed in accordance with previously published guidance (Llewellyn *et al.*, 2011; Mallan *et al.*, 2014).

6.2.5 *Confounder selection*

Potential confounders and covariates were identified a-priori based on relevant clinical information and literature as summarised in Table 6.2. Direct acyclic graphs were drawn including all potential confounders to visualise potential inter-relationships as shown in Figure 6.1. To estimate the effect of early mode of feeding on infant anthropometry, adjustment was made for offspring sex, age at 6 month follow up visit and randomisation to the UPBEAT intervention (Model 1). Further adjustment was made for early pregnancy maternal BMI, ethnicity, socioeconomic deprivation, incidence of GDM and neonatal size at birth (Model 2). To assess the influence of infant appetite and satiety on measures of adiposity and growth at 6 months of age, further adjustment was made for cord blood leptin

concentration and mode of infant feeding (Model 3). Cord blood leptin concentrations have been previously inversely associated with offspring satiety response and adiposity in early infancy (Christou *et al.*, 2001; Bouret, 2010; Boeke *et al.*, 2013). Mode of feeding has shown to be independently associated with growth and adiposity deposition in early life (Owen *et al.*, 2005).

Methods relating to collection of potential confounders and cord blood leptin sample collection and analyses are provided in *Chapter 2: Methods*.

6.2.6 Statistical analysis

A complete case analysis was undertaken. Observations with missing outcome, exposure, and confounder data were excluded from the analysis. Baseline maternal and infant characteristics were summarised by mode of early feeding (exclusive breastfeeding, formula and mixed feeding). Continuous maternal and infant characteristics were summarised by mean (SD) or median (IQR), where appropriate. Binary and categorical variables were summarised by count and percentages. Assessment was undertaken to identify if the distribution of infant anthropometry differed by offspring sex. Comparisons were made between the different modalities of early feeding and measures of infant body composition and anthropometry at 6 months by chi-squared t-test, anova or a Kruskal-Wallis-h-test where appropriate.

To investigate the association between mode of early infant feeding and measures of infant anthropometry, multivariate linear or logistic regression was used as appropriate, with adjustment for potential confounders. To test for potential interactions, Wald test was used to assess whether the relationship between mode of early feeding and infant anthropometry was modified by the timing of introduction of solids. Statistical significance for the interaction tests were defined as $p < 0.05$.

Measures of infant appetite and satiety were summarised using median and IQR. To assess the relationship between mode of early feeding with infant appetite and satiety at 6 months of age, linear regression analysis was undertaken (Llewellyn *et al.*, 2010), with exclusive breastfeeding set as the reference category. To assess the influence of measures of appetite and satiety with infant anthropometry, linear or logistic regression was undertaken as appropriate with adjustment for potential confounders (Model 2 & Model 3).

6.2.6.1 Missing data and sensitivity analysis

To assess for the potential selection bias within this analysis, comparisons were made between mother-offspring pairs included and excluded within this study. A further analysis was undertaken by excluding infants born ≥ 34 weeks and < 37 weeks, in addition to those born < 34 weeks.

From the 698 infants with detailed anthropometric data at 6 months, 47.1% had missing exposure and confounder data, therefore assessment was made for the possibility that missing data resulted in potential selection bias using three complementary methods. Firstly the Little Chi-squared dependent test was undertaken to explore the potential of the data being missing not at random for missing exposure and confounder data in relation to infant outcomes at 6 months (Catalano and Hauguel-De Mouzon, 2011). A second assessment was made, to identify predictors of missingness to determine whether the mechanism of missingness was MCAR or MAR. Thirdly for the primary aim of this study; to assess the mode of feeding on infant growth and body composition, multivariate chain equations was used to impute the missing exposure and confounder data for infants with detailed anthropometric measures at 6 months of age ($n=698$). Assessment was undertaken to determine known predictors of missingness and data was subsequently imputed to create 50 datasets using 10 burn in iterations.

Confounder	Definition	Reasoning
Maternal		
Maternal age	Continuous	Increasing maternal age has been associated with increasing duration of exclusive breastfeeding (Jones <i>et al.</i> , 2011) and reduced abdominal adiposity in infants aged between 19-44 years (Savage <i>et al.</i> , 2013).
Maternal BMI	Continuous	Increasing maternal BMI is an independent risk factor for early introduction of solid food to infants of lean women (Castillo <i>et al.</i> , 2016). Maternal BMI is an independent risk factor for increasing infant adiposity (Lawlor <i>et al.</i> , 2012; Sharp <i>et al.</i> , 2015).
Parity	Binary; 0-Nulip (reference), 1-Multip	Multiparity is associated with increased incidence of breastfeeding and independently associated with higher infant adiposity (Gaillard <i>et al.</i> , 2014a; Hackman <i>et al.</i> , 2015).
Maternal socioeconomic status	Binary; 0-No socioeconomic deprivation (reference), 1-Socioeconomic deprivation	Population level data has identified associations between sociodemographic level and breastfeeding (Oakley <i>et al.</i> , 2013). As discussed in <i>Chapter 1: Introduction</i> , socioeconomic deprivation is associated with an increased risk of childhood obesity (Falconer <i>et al.</i> , 2014).
Maternal ethnicity	Categorical; 0-White (reference), 1-Black, 2-Asian, 3-Other	Maternal ethnicity is strongly associated with infant adiposity. Maternal ethnicity influences the immediate familial environment including feeding practices (Robinson <i>et al.</i> , 2007).
Maternal educational attainment	Binary; 0 <12 years full time education, 1->12 years of full time education (reference)	Reduced educational attainment has been associated with a dose-response relationship with duration of breastfeeding (Lamerz <i>et al.</i> , 2005).
Maternal GDM	Binary; 0- No gestational diabetes (reference), 1- Gestational diabetes	Maternal GDM is associated with infant body composition (Metzger <i>et al.</i> , 2009) and demonstrated to influence mode of feeding (Gunderson, 2007).
Total GWG	Continuous measured in Kg	Observational studies have demonstrated an independent association between maternal total GWG and offspring adiposity (Karachaliou <i>et al.</i> , 2015).
Offspring		
Size at birth	Birthweight/ birthweight z-scores	Size at birth and faster postnatal growth are associated with earlier introduction of solids (Bergmann <i>et al.</i> , 2003). Increased birthweight is associated with larger appetites, greater enjoyment of food and increased responsiveness to food cues in comparison to low birthweight infants (Llewellyn <i>et al.</i> , 2010; Llewellyn <i>et al.</i> , 2011).
Age at follow-up	Continuous variable; measured in days.	Increasing infant age has been associated has consistently been associated with increasing anthropometric measurements (Carberry <i>et al.</i> , 2010).
Infant sex	Binary variable; 0-Female, 1-Male	Adipose tissue is deposited differentially in males and females. Sex differences in glycaemic sensitivity are thought to explain these differences (Regnault <i>et al.</i> , 2013).

Table 6.2: Description and reasoning behind potential confounders and covariates associated with mode of early feeding and infant anthropometry at 6 months of age.

All confounders and their interrelationships were illustrated using direct acyclic graphs and minimal sufficient adjustment was undertaken.

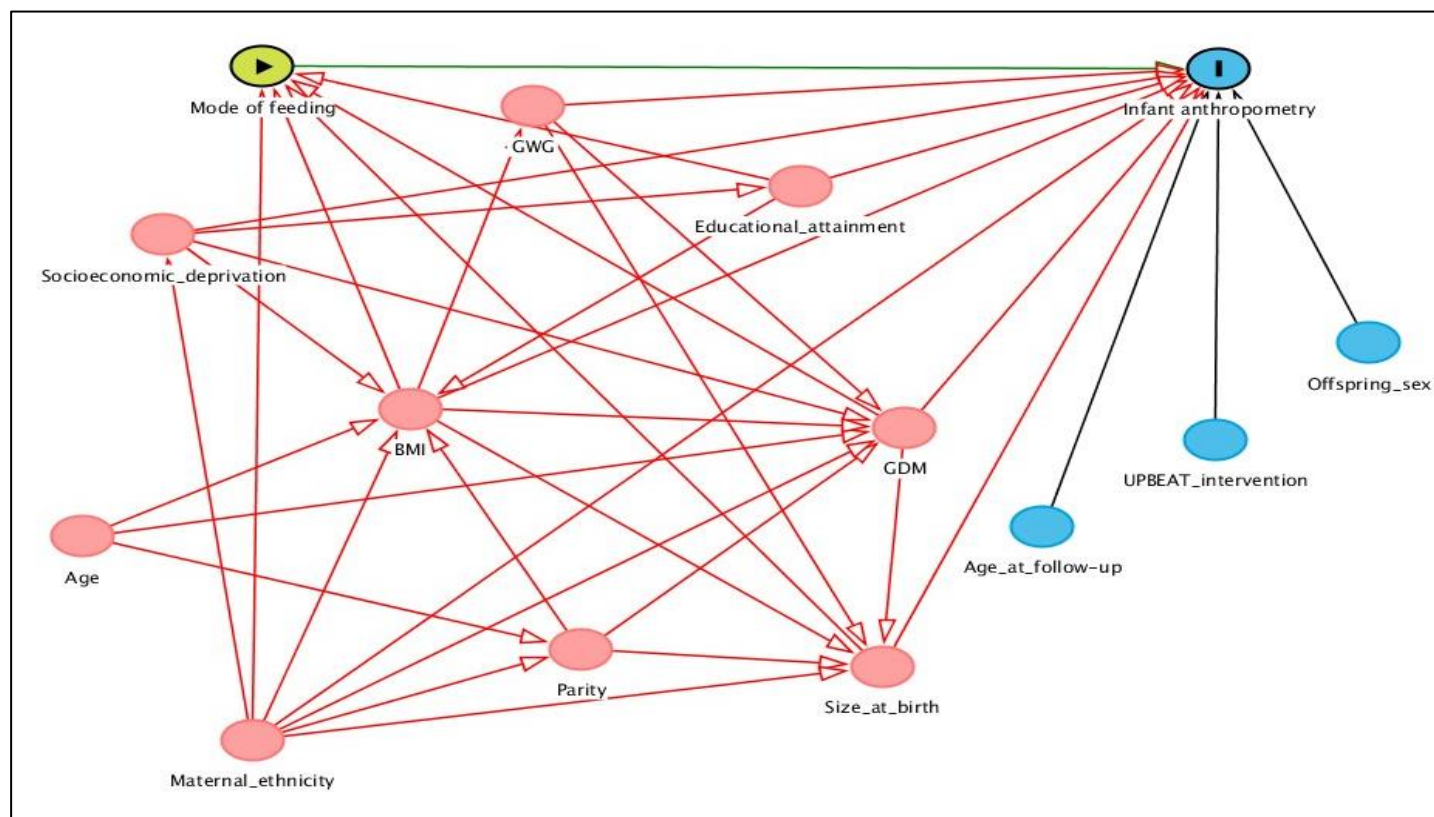


Figure 6.1: Direct acyclic graphs illustrating the interrelationship between potential confounders with early mode of feeding and infant anthropometry at 6 months of age.

The UPBEAT intervention, age at follow-up and infant sex were covariates (blue) within the statistical models. Abbreviations GDM- Gestational diabetes; GWG- Gestational weight gain. Confounders (red) were socioeconomic deprivation, parity, age, ethnicity, early pregnancy BMI, educational attainment, gestational diabetes, gestational weight gain and infant size at birth.

6.3 Results

6.3.1 Demographic characteristics

Of the 1522 live-born neonates, 353 infants were included within this analysis (Figure 6.2).

Infants were excluded for the analysis due to missing early life feeding data (16.6%) and confounder data (32.6%) (Figure 6.2). Infants without anthropometric measurements differed from those with; further details are provided in *Chapter 5: Infant adiposity following a randomised controlled trial of a behavioural intervention in obese pregnancy*.

Of the included infants, 46.7% were exclusively breastfed ≥ 4 months, 45.6% were formula fed and 7.6% were fed a mixture of breast and formula feed. Within this cohort, 46.6% infants were introduced solids <4.6 months of age (Table 6.3). Mothers exclusively breastfeeding were significantly more likely to be older, Caucasian, have a higher BMI, and increased incidence of GDM (Table 6.3). There was no evidence to suggest that the distribution of infant anthropometry differed by offspring sex, therefore reported results were not stratified by infant sex (Figure 6.3).

6.3.2 Association of early mode of feeding and infant anthropometry at 6 months

Univariate analysis comparing mode of early life feeding and infant body composition are summarised in Table 6.4. Following adjustment for maternal and infant confounding, formula feeding was associated with significantly higher weight z-scores and rate of weight gain at 6 months of age in comparison to infants exclusively breastfed (Table 6.5). Formula feeding was also associated with increased odds of catch up growth within the first 6 months of age (Table 6.5). Infants fed a mixture of breast and formula feed had significantly lower arm z-scores at 6 months of age (Table 6.5). Other measures of infant anthropometry did not differ by mode of early life feeding (Table 6.5) following adjustment for potential confounders.

6.3.3 Timing of introduction of solids, mode of early feeding and infant anthropometry at 6 months.

There were no significant differences between mode of early feeding, age at introduction of solids and infant anthropometry at 6 months of age, in offspring born to obese women. (Table 6.6, Figure 6.4, Figure 6.5).

6.3.4 Infant appetite and satiety, mode of early feeding and infant anthropometry at 6 months

Formula feeding in early life was associated with reduced enjoyment of food in comparison to children who were breastfed (Table 6.7). Food responsiveness, general appetite, slowness in eating and satiety responsiveness did not differ between other modes of feeding (Table 6.7).

Measures of general appetite as assessed by the Baby Eating Behaviour Questionnaire were found to be associated with infant subscapular SFT, SSFT, total body fat (%), weight z-scores and catch up growth following adjustment for maternal and offspring confounders (Model 2) (Figure 6.6, Figure 6.7). Following adjustment for cord leptin concentrations and mode of feeding, the associations appeared more apparent (Model 3) (Figure 6.6, Figure 6.7). Reduced satiety responsiveness in contrast was associated with reduced measures SSFT and total body fat, however did not remain significant following adjustment for cord leptin concentrations and mode of early feeding (Model 3) (Figure 6.8, Figure 6.9). There were no associations with measures of enjoyment of food, food responsiveness and slowness of eating with infant growth and adiposity at 6 months of age, in offspring born to obese women (Table 6.8).

6.3.5 *Sensitivity analysis and missing data*

Assessment was made for selection bias and it was found that mothers of infants included within the main analysis were significantly older, were more likely to be nulliparous and more likely to be Caucasian, in comparison to those excluded from the analysis (Table 6.9). There was no difference in the incidence of GDM or total GWG between those included and excluded within the analysis. Infants included did not differ by gestational age at delivery, birthweight or measures of neonatal adiposity including neonatal SSFT or neonatal arm circumference in comparison to those excluded (Table 6.9). However, neonates included had a marginally reduced, but significant, abdominal circumference than those excluded from the analysis at birth (Table 6.9). Exclusion of infants born <37 weeks' gestation, demonstrated no significant differences in the observed associations (Table 6.10).

Using Little's Covariate dependent missing test, there was no evidence of the data being 'missing not at random' (Prob > Chi-square =0.967). Patterns of missing data (including missing exposure and covariate data) were explored. Centre, maternal age, number of years in full time education and gestation at delivery were identified to be significant predictors of missingness, suggesting that the pattern of missing data within this analyses was likely to be MAR (Table 6.11). Maternal early pregnancy BMI, socioeconomic deprivation, total GWG or incidence of GDM were not identified as significant predictors of missingness (Table 6.11). Using multiple imputation by chained equations as a sensitivity analyses, there no was difference in the results obtained from complete-case analysis (Table 6.12).

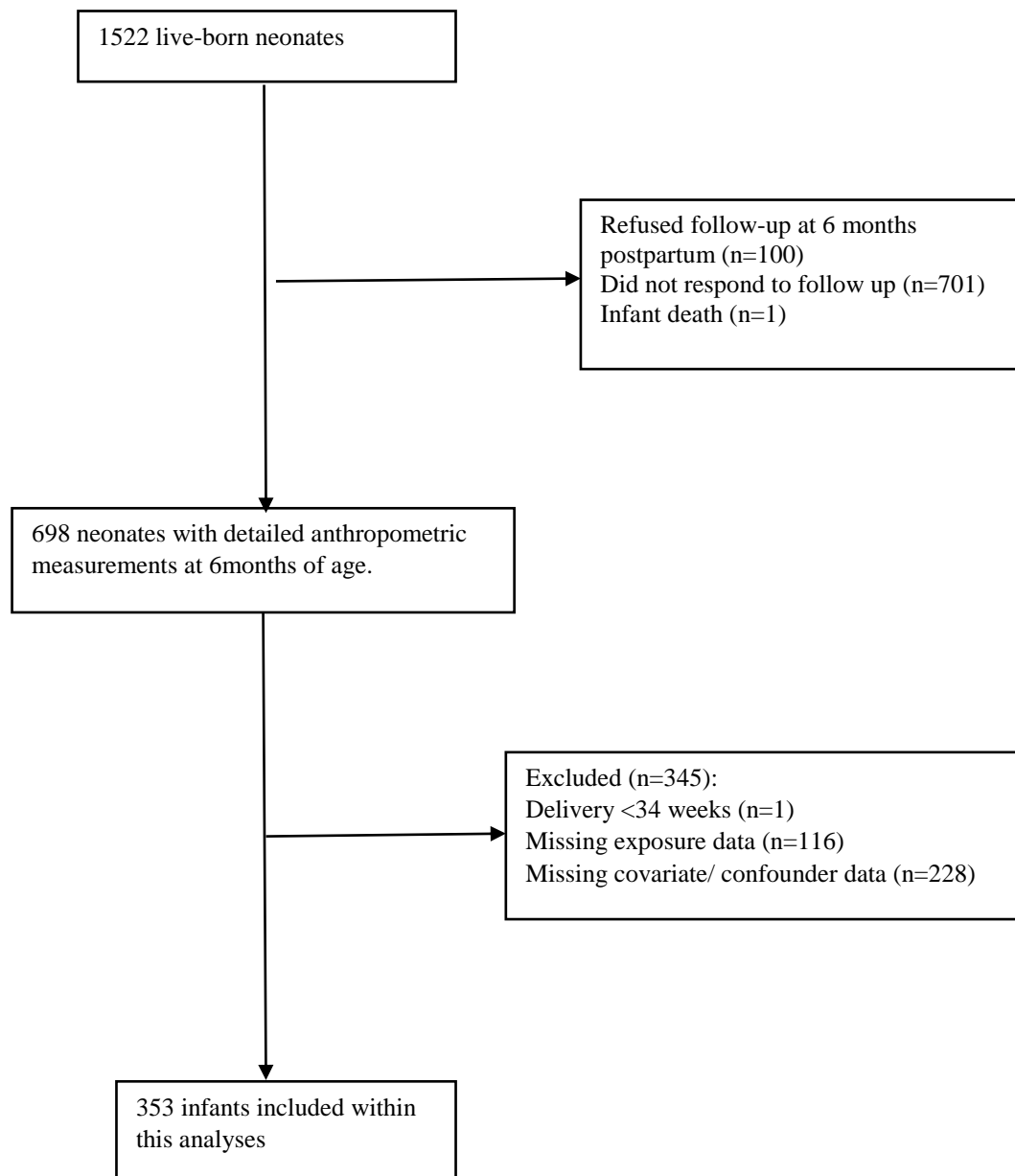


Figure 6.2: Flow diagram of mother-infant pairs included within this analysis.

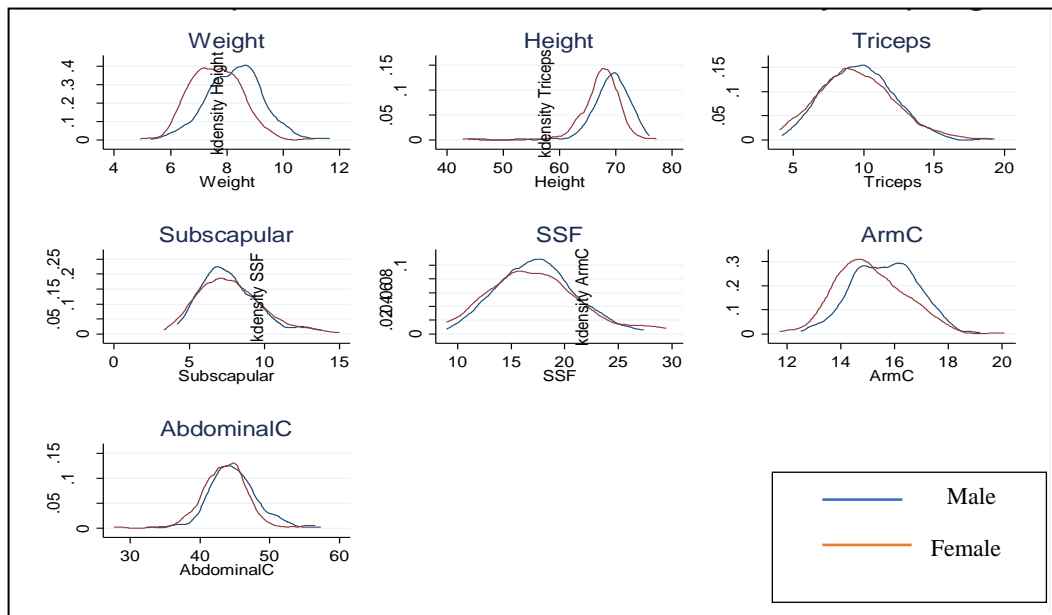


Figure 6.3: Distributional assessment of infant anthropometric measurements at 6 months of age by stratified by offspring sex.

Abbreviations: AbdominalC- Abdominal circumference, ArmC-Arm circumference

		Breast feeding n=165	Formula feeding n=170	Mixed feeding n=29	P-value
		Mean (SD)/ Median (IQR)/ N (%)	Mean (SD)/ Median (IQR)/N (%)	Mean (SD)/Median (IQR)/ N (%)	
Maternal					
Age (years)		32.03 (4.72)	30.92 (5.45)	29.56 (5.55)	0.002
Multiparous		80 (48.48)	68 (40.48)	15 (53.57)	0.220
BMI (kg/m ²)		35.87 (4.96)	37.30 (5.22)	35.06 (5.41)	0.01
Ethnicity	White	101 (61.21)	131 (81.37)	19 (70.37)	<0.001
	Black	40 (24.24)	15 (9.32)	7 (25.93)	0.001
	Asian	7 (4.24)	4 (2.48)	0 (0.00)	0.412
	Other	17 (10.30)	11 (6.83)	1 (3.70)	0.352
Current smoker in early pregnancy		3 (1.82)	5 (3.11)	0 (0.00)	0.054
Socioeconomic deprivation		108 (78.36)	94 (77.05)	17 (85.00)	0.726
Gestational diabetes*		34 (20.61)	62 (38.51)	7 (25.93)	0.002
Gestational weight gain (kg)**		7.19 (4.39)	7.80 (4.75)	8.29 (4.37)	0.339
Neonate					
Gestation at delivery (weeks)		40.29 (39.00 to 41.00)	39.86 (38.43 to 41.71)	39.86 (38.86 to 41.00)	0.077
Birthweight (grams)		3600 (3210 to 3845)	3428 (3155 to 3760)	3360 (3140 to 3660)	0.159
Neonatal sum of skinfold thicknesses (mm)^		10.77 (2.81)	10.92 (2.91)	9.96 (1.78)	0.429
Neonatal abdominal circumference (cm)		32.17 (2.31)	32.30 (2.03)	32.02 (2.31)	0.867
Neonatal arm circumference (cm)		11.46 (0.99)	11.62 (1.00)	11.14 (0.83)	0.158
(log2) Cord blood leptin (ng/ml)		2.85 (0.70)	2.72 (0.72)	2.78 (0.71)	0.49

Table 6.3: Maternal and neonatal demographic, clinical and biochemical characteristics stratified by mode of early feeding; data from the UPBEAT study (n=353)

*Gestational diabetes diagnosed using the International Association of Diabetes in Pregnancy Group's criteria at 24-28 weeks' gestation. **Gestational weight gain defined as total weight gain from calculated pre-pregnancy weight gain to 34-36 weeks' gestation. ^Neonatal sum of skinfolds defined as sum of triceps skinfold thicknesses and subscapular skinfold thicknesses, each measured in triplicates.

	Breastfeeding n=165	Formula feeding n=161	Mixed feeding n=27	p-value
	<i>Mean (SD)/ N (%)</i>	<i>Mean (SD)/ N (%)</i>	<i>Mean (SD)/N (%)</i>	
Triceps skinfold z-scores*	0.05 (1.50)	0.16 (1.38)	0.17 (1.30)	0.79
Subscapular skinfold z-scores*	0.24 (1.45)	0.16 (1.44)	0.30 (1.42)	0.82
Sum of skinfold thickness (mm)	17.30 (4.08)	17.36 (3.94)	17.50 (4.18)	0.97
Total body fat estimation (%) ^	19.75 (5.20)	19.70 (5.06)	19.81 (5.26)	0.99
Weight z-score*	0.15 (1.06)	0.28 (1.01)	0.18 (1.04)	0.52
BMI z-scores*	-0.13 (1.85)	0.06 (1.53)	0.12 (1.44)	0.68
Length z-scores*	0.51 (1.82)	0.53 (1.60)	0.18 (1.49)	0.62
Arm circumference z-scores *	1.07 (1.01)	1.14 (1.02)	1.37 (1.06)	0.39
Weight change (kg/month)	0.63 (0.14)	0.68 (0.13)	0.67 (0.15)	0.02
Length change (cm/month)	2.52 (0.64)	2.64 (0.61)	2.61 (0.54)	0.38
BMI z-scores $\geq 85^{\text{th}}$ *	16 (10.30)	15 (10.00)	4 (16.60)	0.61
BMI z-scores $\geq 95^{\text{th}}$ *	5 (3.11)	8 (5.10)	2 (7.69)	0.47
Catch up growth *	38 (23.60)	56 (35.67)	9 (34.62)	0.06
Catch down growth*	49 (30.43)	36 (23.08)	5 (19.23)	0.23

Table 6.4: Univariate analysis of infant body composition at 6 months of age stratified by mode of early feeding in offspring born to obese women (n=353)

*Infant z-scores calculated using the WHO growth standards; Catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs (WHO, 2006). ^Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988).

		Breastfeeding	Formula feeding (n=161)		Mixed feeding (N=27)	
			Mean difference (95% CI)	p-value	Mean difference (95% CI)	p-value
Triceps SFT z-scores *	Model 1	REF	0.07 (-0.24 to 0.39)	0.64	0.00 (-0.36 to 0.37)	0.98
	Model 2		0.20 (-0.39 to 0.79)	0.51	0.47 (-0.19 to 1.13)	0.16
Subscapular SFT z-scores*	Model 1	REF	-0.10 (-0.41 to 0.21)	0.53	0.21 (-0.14 to 0.57)	0.23
	Model 2		0.03 (-0.55 to 0.62)	0.91	0.37 (-0.27 to 1.01)	0.25
SSFT (mm)	Model 1	REF	-0.04 (-0.92 to 0.84)	0.93	0.37 (-1.27 to 2.00)	0.66
	Model 2		-0.33 (-0.64 to 1.29)	0.51	1.44 (-0.30 to 3.19)	0.10
Total body fat estimation (%) ^	Model 1	REF	0.04 (-1.15 to 1.07)	0.94	0.47 (-1.61 to 2.54)	0.66
	Model 2		-0.44 (-0.78 to 1.65)	0.48	1.84 (-0.37 to 4.04)	0.10
Weight z-scores*	Model 1	REF	0.10 (-0.13 to 0.33)	0.39	0.05 (-0.38 to 0.47)	0.82
	Model 2		0.26 (0.01 to 0.52)	0.04**	0.21 (-0.25 to 0.67)	0.37
BMI z-scores*	Model 1	REF	0.13 (-0.24 to 0.51)	0.49	0.24 (-0.46 to 0.94)	0.50
	Model 2		0.23 (-0.21 to 0.67)	0.31	0.52 (-0.28 to 1.32)	0.20
Length z-scores*	Model 1	REF	-0.01 (-0.39 to 0.38)	0.97	-0.34 (-1.05 to 0.38)	0.35
	Model 2		-0.26 (-1.9 to 0.71)	0.26	-0.30 (-1.11 to 0.51)	0.46
Arm circumference z-scores *	Model 1	REF	0.04 (-0.18 to 0.27)	0.70	0.31 (-0.11 to 0.72)	0.15
	Model 2		0.10 (-0.16 to 0.36)	0.46	0.52 (0.05 to 1.00)	0.03**
Rate of weight gain (kg/ month)	Model 1	REF	0.03 (-0.00 to 0.05)	0.07	0.02 (-0.03 to 0.08)	0.39
	Model 2		0.04 (0.00 to 0.07)	0.04**	0.03 (-0.03 to 0.09)	0.41
Rate of length gain (cm/month)	Model 1	REF	0.07 (-0.10 to 0.25)	0.40	0.05 (-0.26 to 0.36)	0.76
	Model 2		0.15 (-0.07 to 0.37)	0.18	0.01 (-0.35 to 0.37)	0.96
BMI z-scores $\geq 85^{\text{th}}$ *	Model 1	REF	0.99 (0.48 to 2.05)	0.97	1.61 (0.49 to 5.27)	0.43
	Model 2		0.92 (0.35 to 2.40)	0.87	2.28 (0.54 to 9.65)	0.26
BMI z-scores $\geq 95^{\text{th}}$ *	Model 1	REF	1.58 (0.51 to 4.94)	0.43	2.40 (0.44 to 13.05)	0.31
	Model 2		2.45 (0.59 to 10.2)	0.22	2.22 (0.21 to 23.64)	0.51
Catch up growth *	Model 1	REF	1.80 (1.10 to 2.92)	0.02**	1.71 (0.71 to 4.15)	0.24
	Model 2		2.48 (1.31 to 4.71)	0.01**	1.75 (0.59 to 5.25)	0.32
Catch down growth*	Model 1	REF	0.68 (0.41 to 1.13)	0.14	0.55 (0.19 to 1.54)	0.25
	Model 2		0.62 (0.32 to 1.21)	0.16	0.42 (0.10 to 1.75)	0.23

Table 6.5: Multivariate analyses assessing the role of mode of early feeding on measures of infant anthropometry at 6 months of age, in offspring born to obese women (n=353)

**Infant z-scores calculated using the WHO growth standards (WHO, 2006). Catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs; Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates. ^Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988). Model 1- Adjustment made for randomisation to the UPBEAT Intervention, infant sex and infant age at anthropometric measurement. Model 2- Adjustment made randomisation to the UPBEAT Intervention, infant sex and infant age at anthropometric measurement as well as maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth. ** $p < 0.05$*

	Breastfeeding (mean (SD)/ N (%))			Formula (mean (SD)/ N (%))			Mixed (mean (SD)/ N (%))		
	<4 months (n=21)	4-6 months (n=120)	>6 months (n=22)	<4 months (n=40)	4-6 months (n=103)	>6 months (n=18)	<4 months (n=7)	4-6 months (n=19)	>6 months (n=1)
Triceps SFT z-scores*	-0.82 (1.44)	0.21 (1.51)	-0.72 (1.29)	0.10 (1.38)	0.17 (1.42)	0.22 (1.14)	0.08 (1.45)	0.33 (1.17)	-
Subscapular z-scores*	0.10 (1.46)	0.27 (1.43)	0.23 (1.58)	-0.05 (1.41)	0.18 (1.43)	0.51 (1.61)	1.00 (1.12)	0.19 (1.43)	-
SSFT (mm)	15.55 (3.72)	17.62 (4.14)	17.06 (3.77)	16.94 (3.70)	17.39 (4.02)	18.13 (4.05)	18.58 (4.86)	17.49 (3.89)	-
Total body fat estimation (%)	17.48 (4.82)	20.17 (5.30)	19.41 (4.60)	19.25 (4.68)	19.71 (5.20)	20.66 (5.19)	21.57 (6.54)	19.68 (4.69)	-
Weight z-scores*	0.03 (0.79)	0.22 (1.08)	-0.07 (1.15)	0.23 (1.05)	0.29 (0.98)	0.36 (1.18)	-0.11 (1.14)	0.32 (1.03)	-
BMI z-scores*	-0.51 (1.07)	-0.04 (2.02)	-0.28 (1.26)	0.17 (2.00)	0.00 (1.37)	-0.36 (1.28)	-0.65 (1.11)	0.40 (1.50)	-
Length z-scores*	0.72 (1.40)	0.53 (1.97)	0.22 (1.25)	0.28 (2.15)	0.53 (1.42)	1.11 (1.25)	0.68 (0.86)	0.06 (1.66)	-
Arm circumference z-scores	0.60 (1.06)	1.17 (1.00)	0.95 (1.08)	1.02 (1.03)	1.17 (1.03)	1.21 (0.96)	1.24 (1.08)	1.48 (1.06)	-
Rate of weight change (kg/month)	0.65 (0.15)	0.63 (0.14)	0.63 (0.14)	0.68 (0.13)	0.67 (0.13)	0.69 (0.14)	0.66 (0.11)	0.68 (0.16)	-
Rate of length change (cm/month)	2.90 (0.25)	2.48 (0.69)	2.52 (0.43)	2.62 (0.79)	2.57 (0.65)	2.84 (0.52)	2.81 (0.19)	2.54 (0.62)	-
BMI ≥95 th centile*	0 (0.0)	5 (4.2)	0 (0.0)	3 (7.9)	4 (3.9)	1 (6.3)	0 (0.0)	2 (10.5)	0 (0.0)
BMI ≥85 th centile*	0 (0.0)	12 (10.4)	4 (18.2)	2 (5.7)	12 (12.1)	1 (6.7)	0 (0.0)	4 (23.5)	0 (0.0)
Catch up growth	7 (36.8)	26 (21.7)	5 (22.7)	16 (42.1)	35 (34.0)	5 (31.3)	2 (33.3)	7 (36.8)	0 (0.0)
Catch down growth	5 (26.3)	37 (30.8)	7 (31.8)	6 (15.7)	28 (27.5)	2 (12.5)	0 (0.0)	4 (21.1)	0 (0.0)

Table 6.6: Differences in infant anthropometry stratified by mode of early life feeding and age at introduction of solids in offspring born to obese women (n=353).

*Infant z-scores calculated using the WHO growth standards(WHO, 2006). Catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs; Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates. ^Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988). Wald test for 3-way interaction $p>0.05$ for all infant anthropometric measures at 6 months of age. Adjustment made for randomisation to the UPBEAT intervention, infant sex, infant age at anthropometric measurement collection, maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth.

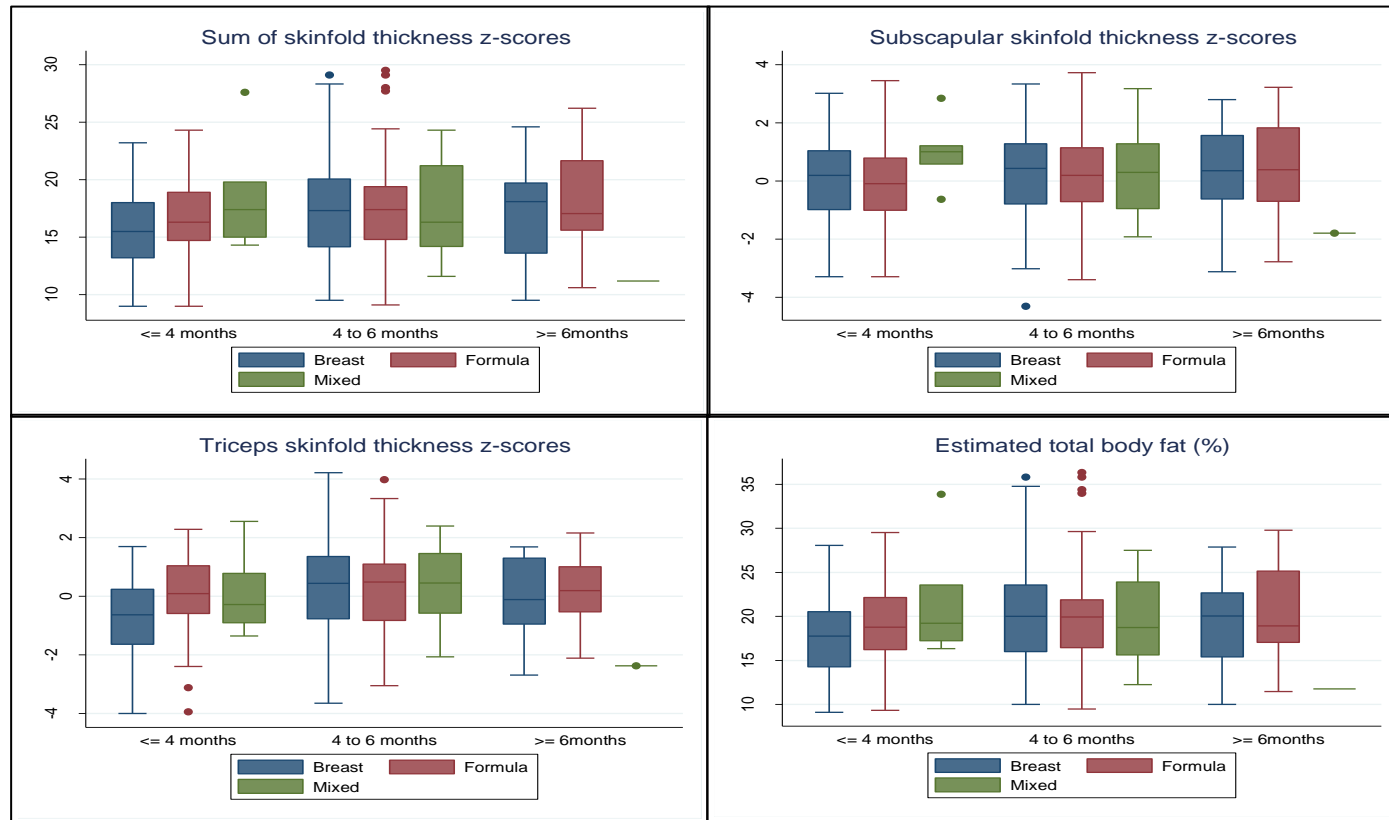


Figure 6.4: The association between mode of early feeding, timing of introduction of solid foods and infant adiposity at 6 months of age, in offspring born to obese women (n=353)

Wald test for interaction $p > 0.05$. Breastfeeding and introduction of solids ≥ 4.6 months was treated the reference category within the analysis. Infant z-scores calculated using the WHO growth standards (WHO, 2006); definitions of change in weight > 0.67 SDs; Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates. Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988). Adjustment made for randomisation to the UPBEAT Intervention, infant sex and infant age at anthropometric measurement as well as maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth.

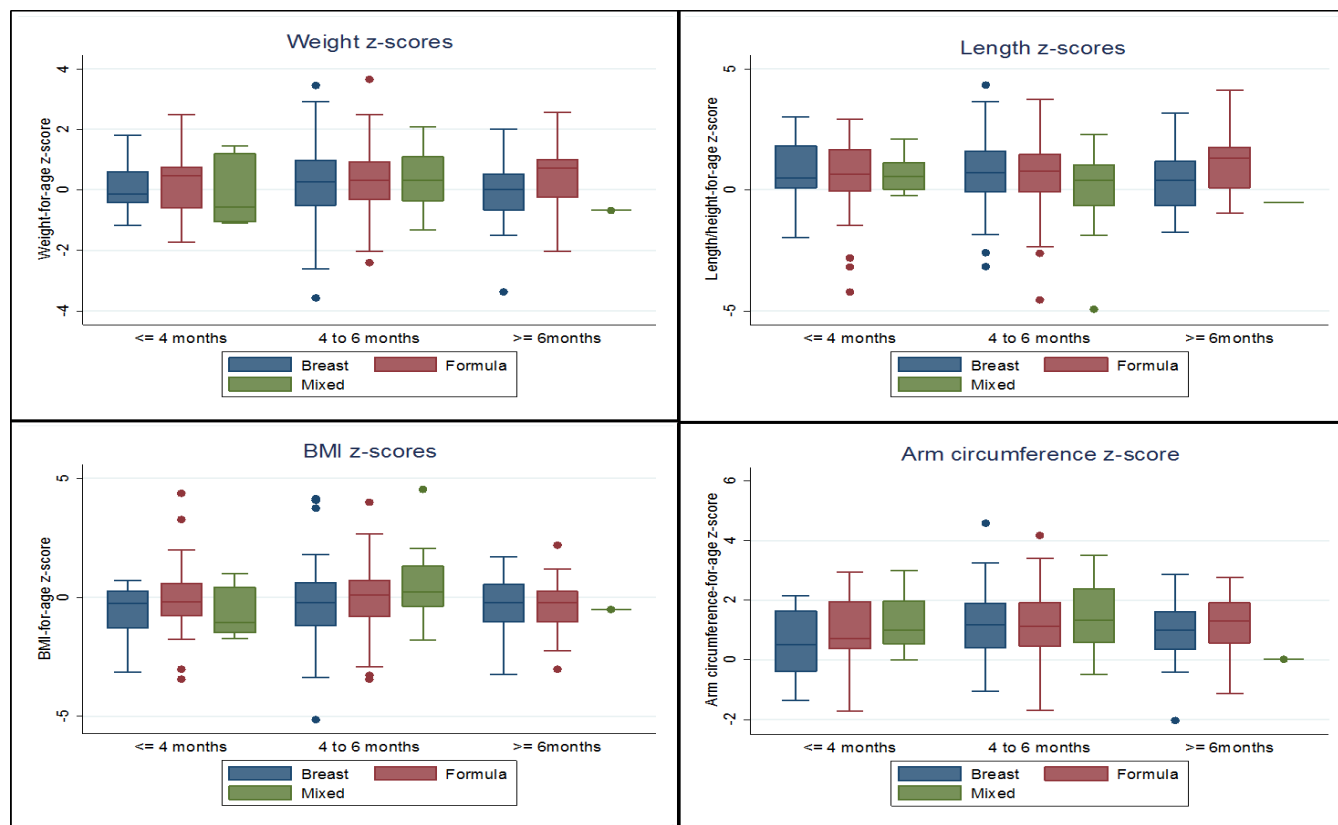


Figure 6.5: The association between mode of early feeding, timing of introduction of solid foods and measures of infant growth at 6 months of age, in offspring born to obese women (n=353).

Wald test for interaction $p > 0.05$. Breastfeeding and introduction of solids ≥ 4.6 months was treated the reference category within the analysis. Infant z-scores calculated using the WHO growth standards (WHO, 2006); definitions of change in weight > 0.67 SDs; Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates. Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988). Adjustment made for randomisation to the UPBEAT Intervention, infant sex and infant age at anthropometric measurement as well as maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth.

	Formula (N=161)				Mixed (N=27)			
	<i>Coef*</i>	<i>95% CI</i>		<i>p-value</i>	<i>Coef*</i>	<i>95% CI</i>		<i>p-value</i>
		<i>LL</i>	<i>UL</i>			<i>LL</i>	<i>UL</i>	
Enjoyment of food	-0.751	-1.235	-0.267	0.002	-0.909	-1.974	0.156	0.094
Food responsiveness	0.365	-0.745	1.476	0.518	1.583	-4.222	3.588	0.121
General appetite	-0.180	-0.441	0.081	0.176	0.043	-0.399	0.486	0.847
Slowness in eating	-0.035	-0.578	0.507	0.898	0.380	-0.618	1.379	0.455
Satiety responsiveness	0.371	-0.161	0.902	0.171	0.467	-0.607	1.541	0.393

Table 6.7: Measures of infant appetite and satiety at 6 months of age by mode of early feeding in offspring born to obese women (n=353).

Data obtained from the validated Baby Eating Behaviour Questionnaire (Llewellyn et al., 2011). Adjustment made for randomisation to the UPBEAT intervention, infant sex, infant age at anthropometric measurement as well as maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth.

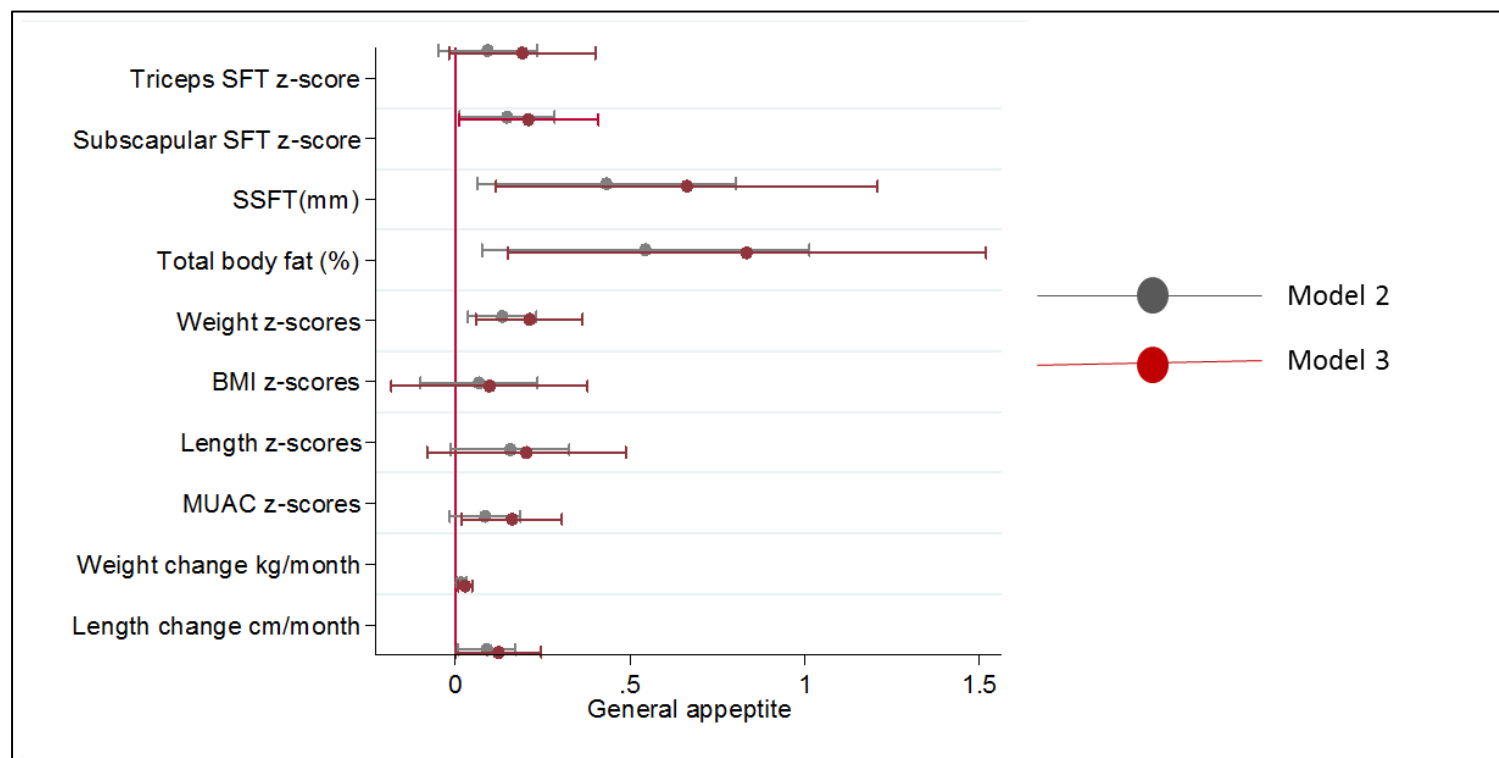


Figure 6.6: Associations between measures of general appetite with infant adiposity and anthropometry at 6 months of age, in offspring born to obese women (n=353).

Regression coefficients and 95% confidence intervals plotted. Model 2: adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex and age at 6 month follow-up visit. Model 3- adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex, cord blood leptin, age at 6 month follow-up and mode of early feeding. Abbreviations BMI-Body mass index, MUAC-Mid arm upper circumference, SFT-skinfold thickness, SSFT-sum of skinfold thicknesses.

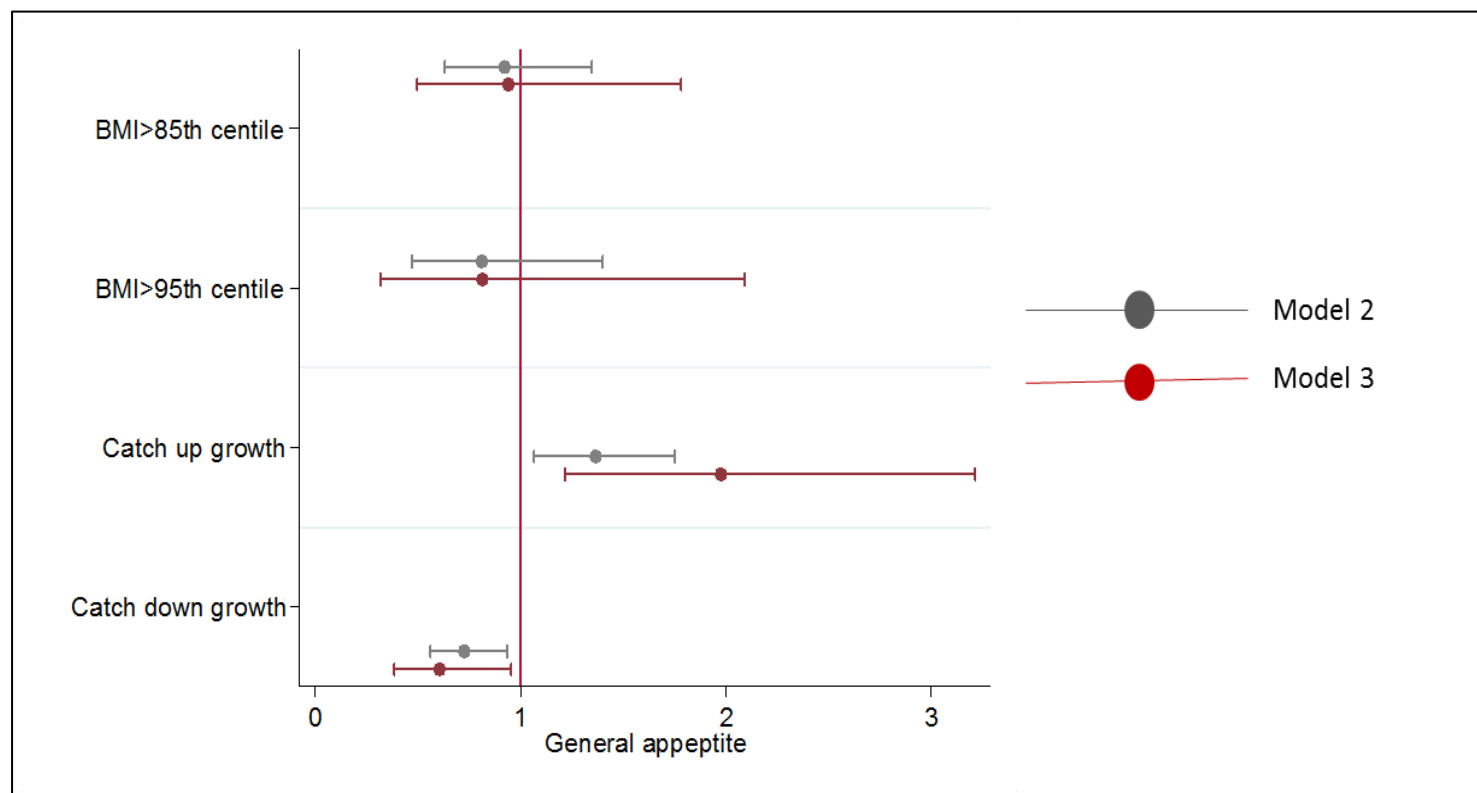


Figure 6.7: Associations between measures of general appetite with infant obesity and growth at 6 months of age, in offspring born to obese women (n=353).

Odds ratio and 95% confidence intervals plotted. Model 2: adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, and socioeconomic deprivation, and ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex and age at 6 month follow-up visit. Model 3- adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex, cord blood leptin, age at 6 month follow-up and mode of early feeding. Abbreviations BMI-Body mass index, catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs

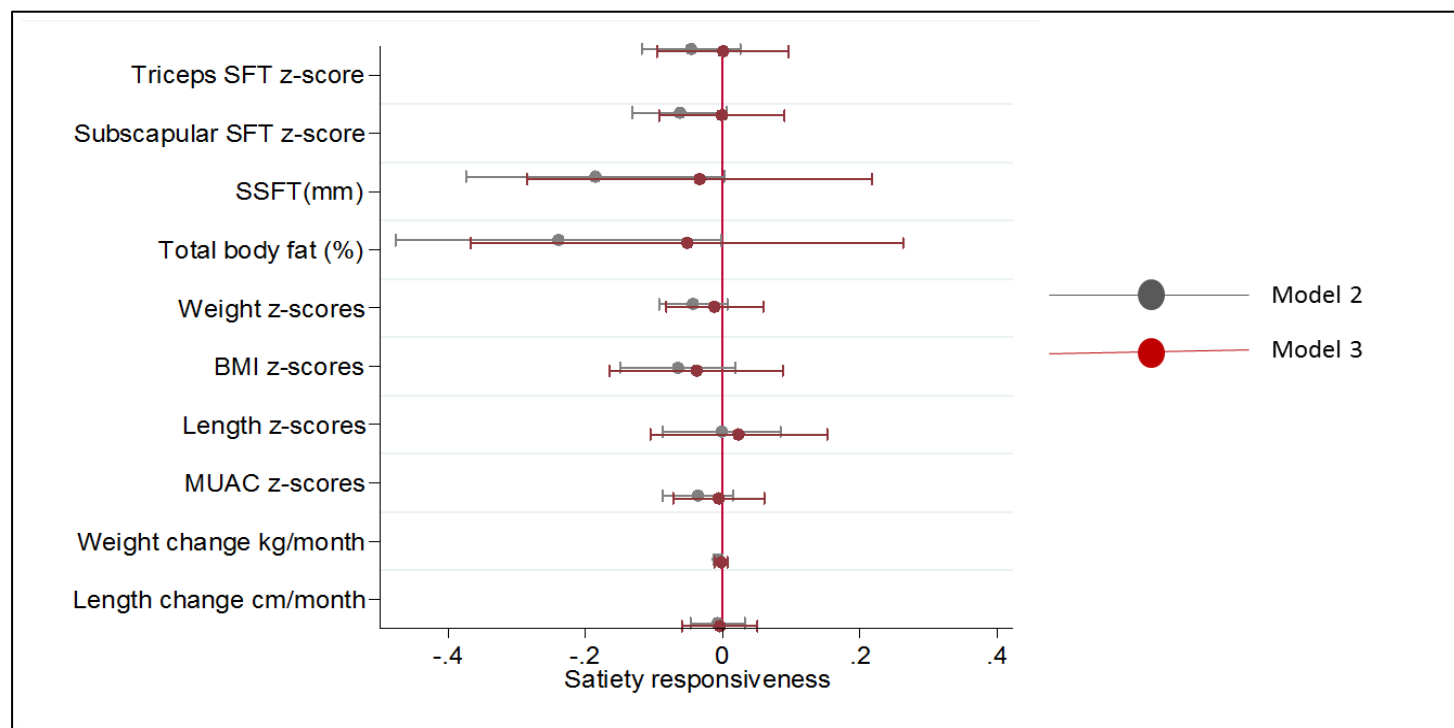


Figure 6.8: Associations between measures of satiety responsiveness with infant adiposity and anthropometry at 6 months of age, in offspring born to obese women (n=353).

Regression coefficients and 95% confidence intervals plotted. Model 2: adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex and age at 6 month follow-up visit. Model 3- adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex, cord blood leptin, age at 6 month follow-up and mode of early feeding. Abbreviations BMI-Body mass index, MUAC-Mid arm upper circumference, SFT-skinfold thickness, SSFT-sum of skinfold thicknesses.

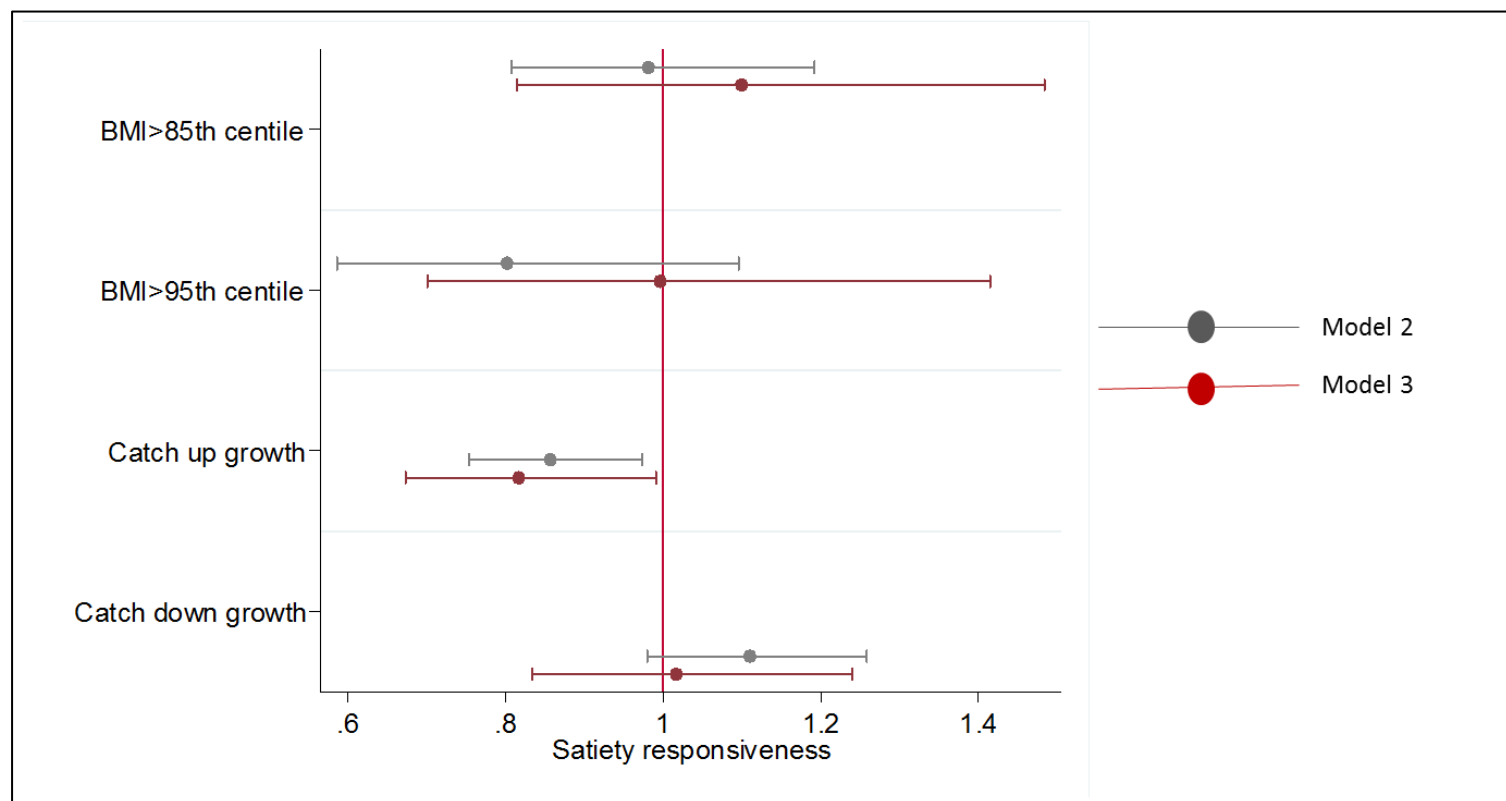


Figure 6.9: Associations between measures of satiety responsiveness with infant obesity and growth at 6 months of age, in offspring born to obese women (n=353).

Odds ratio and 95% confidence intervals plotted. Model 2: adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex and age at 6 month follow-up visit. Model 3- adjustment made for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, diagnosis of gestational diabetes, offspring birthweight, sex, cord blood leptin, age at 6 month follow-up and mode of early feeding. Abbreviations BMI-Body mass index, catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs

	Enjoyment of food	Food responsiveness	Slowness in eating
	<i>Mean difference (95% CI)/ Odds ratio (95% CI) †</i>	<i>Mean difference (95% CI)/ Odds ratio (95% CI) †</i>	<i>Mean difference (95% CI)/ Odds ratio (95% CI) †</i>
Triceps skinfold z-scores *	-0.04 (-0.19 to 0.10)	0.02 (-0.03 to 0.07)	-0.04 (-0.15 to 0.06)
Subscapular skinfold thickness z-scores*	-0.05 (-0.19 to 0.09)	0.03 (-0.01 to 0.08)	-0.01 (-0.11 to 0.09)
Sum of skinfold thickness (mm)**	-0.10 (-0.48 to -0.28)	0.08 (-0.47 to 0.21)	-0.10 (-0.37 to 0.17)
Total body fat estimation (%) ^	-0.12 (-0.60 to 0.36)	0.10 (-0.05 to 0.26)	-0.13 (-0.47 to 0.21)
Weight z-scores*	-0.07 (-0.18 to 0.03)	0.02 (-0.02 to 0.06)	-0.02 (-0.09 to 0.06)
BMI z-scores *	-0.06 (-0.26 to 0.13)	-0.08 (-0.07 to 0.06)	-0.12 (-0.26 to 0.02)
Length z-scores*	-0.05 (-0.25 to 0.15)	0.03 (-0.03 to 0.10)	0.08 (-0.05 to 0.22)
Arm circumference z-scores *	-0.06 (-0.16 to 0.04)	0.01 (-0.03 to 0.04)	-0.03 (-0.10 to 0.04)
Weight change (kg/month)	-0.01 (-0.02 to 0.00)	0.00 (-0.00 to 0.01)	-0.00 (-0.01 to 0.01)
Length change (cm/month)	-0.04 (-0.12 to 0.05)	0.15 (-0.01 to 0.43)	0.04 (-0.01 to 0.10)
BMI z-scores ≥85 th centile	0.86 (0.63 to 1.18)	1.03 (0.88 to 1.20)	0.72 (0.51 to 1.04)
BMI z-scores ≥95 th centile	1.02 (0.51 to 2.06)	0.78 (0.57 to 1.10)	0.57 (0.29 to 1.11)
Catch up growth**	0.98 (0.76 to 1.26)	1.13 (0.98 to 1.26)	0.88 (0.71 to 1.07)
Catch down growth**	1.10 (0.78 to 1.55)	0.98 (0.90 to 1.08)	1.01 (0.81 to 1.25)

Table 6.8: Associations between measures of infant appetite including measures of enjoyment of food, food responsiveness and slowness in eating with infant adiposity and anthropometry at 6 months of age, in offspring born to obese women (n=353).

No significant associations were observed between measures of enjoyment of food, food responsiveness and slowness of eating with infant anthropometry at 6 months of age. Data obtained from the validated Baby Eating Behaviour Questionnaire (Llewellyn et al., 2011). *Infant z-scores calculated using the WHO growth standards (WHO, 2006); **Catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs; Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates. ^Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988). † Analyses adjusted for randomisation to the UPBEAT intervention, maternal early pregnancy BMI, socioeconomic deprivation, ethnicity, and diagnosis of gestational diabetes, offspring birthweight, sex, and cord blood leptin, age at 6 month follow-up and mode of early feeding, cord leptin and mode of early feeding.

	Included	Excluded	P-value
	<i>Mean (SD)/ Median (IQR)/ N (%)</i> N=353	<i>Mean (SD)/ Median (IQR)/ N (%)</i> N=1167	
Age (years)	31.33 (5.16)	30.20 (5.58)	<0.001
Multiparous	162 (45.8)	697 (59.8)	<0.001
BMI (kg/m ²)	36.45 (5.16)	36.25 (4.65)	0.508
Ethnicity			
White	251 (70.9)	706 (60.5)	<0.001
Black	63 (17.8)	325 (27.9)	<0.001
Asian	11 (3.1)	80 (6.9)	0.012
Other	29 (8.2)	55 (4.7)	0.013
Current smoker in early pregnancy	8 (2.3)	97 (8.3)	<0.001
Socioeconomic deprivation	217 (78.3)	790 (81.0)	0.338
Gestational diabetes*	103 (29.1)	274 (28.4)	0.794
Gestational weight gain (kg)**	7.54 (4.55)	7.48 (4.54)	0.838
Gestation at delivery (weeks)	39.71 (38.71 to 40.86)	40.00 (38.86 to 40.86)	0.032
Birthweight (grams)	3500 (3165 to 3806)	3450 (3100 to 3790)	0.126
Neonatal sum of skinfold thicknesses (mm)^	10.77 (2.78)	11.02 (2.55)	0.320
Neonatal abdominal circumference (cm)	32.22 (2.18)	32.68 (1.98)	0.016
Neonatal arm circumference (cm)	11.51 (0.99)	11.46 (1.15)	0.651

Table 6.9: Comparison of baseline maternal and neonatal demographic, clinical, and anthropometric characteristics between those included (n=353) and excluded (n=1167) from the analysis.

**Gestational diabetes diagnosed using the International Association of Diabetes in Pregnancy Group's criteria at 24-28 weeks' gestation. **Gestational weight gain defined as total weight gain from calculated pre-pregnancy weight gain to 34-36 weeks' gestation. ^Neonatal sum of skinfolds defined as sum of triceps skinfold thicknesses and subscapular skinfold thicknesses, each measured in triplicates.*

	Breastfeeding	Formula feeding	Mixed feeding
		Mean difference/ Odds ratio (95% CI)	Mean difference/ Odds ratio (95% CI)
Triceps skinfold z-scores*	REF	-0.02 (-0.39 to 0.35)	0.47 (-0.19 to 1.14)
Subscapular skinfold z-scores*	REF	0.21 (-0.15 to 0.57)	0.37 (-0.27 to 1.01)
Sum of skinfold thickness (mm)**	REF	0.27 (-0.71 to 1.26)	1.44 (-0.32 to 3.20)
Weight z-scores*	REF	0.27 (0.01 to 0.52)	0.23 (-0.23 to 0.69)
BMI z-scores*	REF	0.21 (-0.24 to 0.66)	0.54 (-0.27 to 1.34)
Length z-scores*	REF	0.29 (-0.17 to 0.74)	-0.28 (-1.10 to 0.54)
Total body fat estimation (%) ^	REF	0.37 (-0.88 to 1.61)	1.83 (-0.39 to 4.05)
Arm circumference z-scores *	REF	0.09 (-0.18 to 0.35)	0.54 (0.6 to 1.01)
Weight change (kg/ month)	REF	0.04 (0.01 to 0.07)	0.03 (-0.03 to 0.09)
Length change (cm/month)	REF	0.16 (-0.06 to 0.38)	0.01 (-0.36 to 0.38)
Catch up growth	REF	2.40 (1.40 to 4.12)	1.24 (0.46 to 3.31)
Catch down growth	REF	0.67 (0.38 to 1.19)	0.45 (0.13 to 1.50)

Table 6.10: Sensitivity analysis of removal of infants born >34 weeks' gestation and ≤37 weeks' gestation (n=8 excluded).

*Infant z-scores calculated using the WHO growth standards (WHO, 2006). **Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates. ^Infant total body fat estimation calculated sex-specific, validated equations (Slaughter et al., 1988). †Analyses adjusted for randomisation to the UPBEAT Intervention, infant sex and infant age at anthropometric measurement as well as maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth.

		Missing data	Complete data	Comparison
		N (%), Mean (SD)	N (%), Mean (SD)	
		N=345	N=353	
Centre	St Thomas’	100 (28.9)	107 (30.4)	0.021
	King’s College Hospital	54 (15.9)	53 (14.8)	
	Newcastle	49 (14.2)	71 (20.2)	
	Glasgow	107 (30.9)	77 (21.9)	
	Manchester	13 (3.8)	28 (8.0)	
	Bradford	5 (1.4)	7 (2.0)	
	Sunderland	8 (2.3)	5 (1.4)	
	St Georges’	9 (2.6)	5 (1.4)	
Maternal demographic characteristics				
Age (years)		30.1 (5.70)	31.6 (5.05)	0.001
Multiparity		181 (52.3)	162 (46.0)	0.097
Ethnicity	White	249 (72.0)	249 (70.7)	0.72
	Black	71 (20.5)	63 (17.9)	
	Asian	14 (4.0)	11 (3.1)	
	Other	12 (3.5)	29 (8.2)	
Socioeconomic deprivation		200 (84.4)	217 (78.9)	0.11
Number of years in full time education		14.5 (2.9)	15.7 (2.7)	<0.001
Maternal anthropometry				
Early pregnancy BMI (kg/m²)		36.1 (4.5)	36.4 (5.1)	0.34
Sum of skinfold thickness (mm)	15-18 weeks’ gestation	123.8 (27.0)	122.7 (26.6)	0.58
	27-28 ⁺⁶ weeks’ gestation	126.6 (25.1)	124.0 (27.0)	0.19
	34-36 weeks’ gestation	123.4 (26.7)	122.8 (27.0)	0.79
Total gestational weight gain (kg)**		7.2 (4.6)	7.6 (4.5)	0.24
Antenatal clinical history				
Diagnosis of GDM*		96 (28.9)	103 (29.3)	0.92
Pre-eclampsia		12 (3.5)	10 (2.9)	0.63
Birth outcomes				
Mode of delivery	Vaginal	165 (47.7)	170 (48.3)	0.32
	Instrumental	51 (14.7)	369 (11.1)	
	C-section	130 (37.6)	143 (40.6)	
Postpartum haemorrhage (>1l)		47 (13.7)	59 (16.9)	0.24
Neonatal sex	Male	173 (50.0)	182 (51.7)	0.65
	Female	173 (50.0)	170 (48.3)	
Gestation at delivery (weeks)		39.4 (2.3)	39.9 (1.5)	0.004
Birthweight (kg)		3.42 (0.61)	3.59 (0.52)	0.09
Neonatal sum of skinfold thicknesses (mm)^		10.8 (2.8)	11.0 (2.5)	0.44
Admission to NICU		33 (9.5)	22 (6.3)	0.11

Table 6.11: Predictors of missing exposure (mode of infant feeding) and maternal covariate data in infants with detailed anthropometric data at 6 months of age.

**Gestational diabetes diagnosed using the International Association of Diabetes in Pregnancy Group's criteria at 24-28 weeks' gestation. **Gestational weight gain defined as total weight gain from calculated pre-pregnancy weight gain to 34-36 weeks' gestation. ^Neonatal sum of skinfolds defined as sum of triceps skinfold thicknesses and subscapular skinfold thicknesses, each measured in triplicates*

	Breastfeeding (46.4%)	Formula feeding (46.0%)	Mixed feeding (7.6%)
		Mean difference/ Odds ratio (95% CI) **	Mean difference/ Odds ratio (95% CI) **
Triceps SFT z-scores*	REF	0.13 (0.18 to 0.43)	0.31 (-0.23 to 0.86)
Subscapular SFT z-scores*	REF	0.03 (-0.23 to 0.34)	0.12 (-0.43 to 0.66)
SSFT (mm)	REF	-0.27 (-1.11 to 0.57)	0.42 (-1.05 to 1.89)
Total body fat estimation (%)	REF	-0.34 (-1.40 to 0.72)	0.54 (-1.33 to 2.40)
Weight z-scores*	REF	0.25 (0.06 to 0.44) ^	0.11 (-0.25 to 0.46)
BMI z-scores*	REF	-0.00 (-0.34 to 0.33)	0.07 (-0.55 to 0.69)
Length z-scores*	REF	-0.37 (-0.49 to 0.75)	-0.04 (-1.36 to 0.38)
Arm circumference z-score	REF	0.21 (-0.11 to 0.52)	0.74 (-0.11 to 1.05)
Rate of weight gain (kg/month)	REF	0.03 (0.01 to 0.06) ^	0.01 (-0.04 to 0.06)
Rate of length gain (cm/month)	REF	0.31 (-0.14 to 0.75)	0.27 (-0.07 to 0.61)
BMI z-scores $\geq 85^{\text{th}}$ *	REF	1.06 (0.54 to 2.08)	1.89 (0.63 to 5.68)
BMI z-scores $\geq 95^{\text{th}}$ *	REF	1.10 (0.40 to 3.07)	1.62 (0.32 to 8.26)
Catch up growth	REF	2.06 (1.31 to 3.26) ^	1.15 (0.49 to 2.70)
Catch down growth	REF	0.78 (0.49 to 1.26)	0.66 (0.26 to 1.67)

Table 6.12: Sensitivity analyses assessing the role of mode of early feeding on measures of infant anthropometry at 6 months of age., in offspring born to obese women (n=353) using multiple imputation.

*Infant z-scores calculated using the WHO growth standards (WHO, 2006); Catch up and catch down growth defined using the WHO definitions of change in weight >0.67 SDs; Infant sum of skinfold thicknesses calculated as the addition of subscapular and triceps skinfolds thicknesses, each measured in triplicates.

^Infant total body fat estimation calculated using sex-specific, validated equations (Slaughter et al., 1988)..

Multiple imputation methodology; data was imputed to create 50 datasets using 10 burn-in iterations for missing mode of feeding (exposure) and covariate data for infants with detailed anthropometry at 6 months of age (n=698, 49.2% missing) using centre, maternal age, trial entry BMI, parity, smoking status, randomisation allocation, ethnicity, socioeconomic deprivation, diagnosis of gestational diabetes, total gestational weight gain, sum of skinfold thicknesses at 15-18⁺⁶, 27-28⁺⁶ and 34-36 weeks' gestation, mode of delivery, gestation at delivery, admission to neonatal intensive care, neonatal sex and offspring age at 6 month visit. Multivariate regression analyses (adjusted for potential confounders) was performed and effect estimates were estimate on pooled datasets using mean difference (continuous outcomes) and odds ratio (binary outcomes). **Adjustment made randomisation for the UPBEAT Intervention, infant sex and infant age at anthropometric measurement as well as maternal early pregnancy BMI, ethnicity, socioeconomic deprivation, gestational diabetes and infant size at birth. ^ $p < 0.05$

6.4 Discussion

6.4.1 Summary of overall findings

The increasing incidence of maternal obesity is of public health concern, not only because of the immediate adverse health outcomes, but also due to the possible transgenerational association with childhood obesity (Poston *et al.*, 2016). As the trajectory of later obesity is also thought to be determined in early infancy, targeted interventions within this period may have a major influence on curbing the escalating incidence (Giles *et al.*, 2015).

Although breastfeeding has been demonstrated to be protective against childhood obesity (Armstrong and Reilly, 2002; Arenz *et al.*, 2004; Smith, 2012; Robinson *et al.*, 2015b), obese mothers have reduced rates of initiation and duration of exclusive breastfeeding (as assessed as a continuous measure) (Bever Babendure *et al.*, 2015).

This investigation addressed the influence of mode of early feeding with infant anthropometry and demonstrated a protective influence of exclusive breastfeeding on the infant. Exclusive breastfeeding ≥ 4 months was found to be protective against linear increases in weight z-scores, velocities of weight gain and catch up growth but not with measures of adiposity in comparison to infants' formula or mixed fed. Measures of general appetite in early infancy were associated with measures of adiposity, weight and catch up growth independent of cord blood leptin concentrations and mode of early feeding.

6.4.2 Association between breastfeeding and infant anthropometry, body composition at 6 months of age.

To our knowledge, this is the first study addressing breastfeeding and infant adiposity in obese women. Within this high risk group, this study demonstrated that exclusive breastfeeding ≥ 4 months in obese women is associated with reduced measures of infant weight at 6 months of age and weight gain velocity, independent of maternal early

pregnancy BMI and GDM. A protective effect of breastfeeding and later childhood obesity was initially proposed by Kramer et al (Kramer *et al.*, 2004). To date, studies have demonstrated small improvements in measures of growth in offspring born to women of heterogeneous BMI. Determining the true effect of breastfeeding on later childhood obesity, is difficult due to the large number of confounders. For example, socioeconomic deprivation is known to influence duration of exclusive breastfeeding as well as dietary composition in early life (Young *et al.*, 2012). Meta-analyses suggest small reductions in childhood obesity (Owen *et al.*, 2005; Yan *et al.*, 2014). A dose response relationship has been suggested between duration of exclusive breastfeeding for >1 month and an associated 30% risk reduction of obesity in adolescence (Harder *et al.*, 2005). However, the majority of studies have assessed older children, than investigated in the present study; therefore, direct comparisons in relation to effect estimates with this study are difficult to make.

This analysis demonstrated reduced catch-up and weight gain velocities in breastfed infants which are comparable to studies in women of heterogeneous BMI and this may benefit in relation to later obesity risk. Rapid weight gain and catch up growth in early infancy are associated with an increased risk of later childhood obesity (Baird *et al.*, 2005; Ong and Loos, 2006). Within the ALSPAC cohort, catch up growth from birth to two years was associated with increased visceral fat mass at 5 years of age (Ong *et al.*, 2000). In another longitudinal study undertaken in the prospective Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) cohort, it was observed that in boys alone breastfed for <16 weeks, born to overweight women there was significantly greater postnatal growth up to 7 years of age in comparison to those breastfed >17 weeks (Buyken *et al.*, 2008). Together these studies and the UPBEAT study, suggest that breastfeeding may have the potential to offset the ‘*programming*’ effect of childhood obesity, through modification of early growth velocities.

6.4.3 Role of timing of introduction of solids and infant anthropometry at 6 months

This study demonstrated limited adherence to current WHO breastfeeding guidelines amongst obese women (exclusive breastfeeding in 46.7% and introduction of solids ≥ 6 months of age in 12.1% of the included cohort), highlighting the need for targeted interventions within the postpartum period for obese women to promote exclusive breastfeeding and later introduction of solids.

There was no association between mode of early life feeding, further stratified by timing of introduction to solids with infant anthropometry at 6 months of age, born to obese women. This finding is supported by a previous observational study demonstrating no association between the timing of solid food introduction and later child adiposity at 6 and 12 months of age (Huh *et al.*, 2011) in offspring born to women of heterogeneous BMI. However a recent systematic review identified that early introduction of solids (≤ 4 months of age) was associated with a small increased risk of developing overweight/ obesity in childhood (Daniels *et al.*, 2015). Early introduction of solids is associated with increased energy consumption, and also provides vital micronutrients including vitamin D, iron, zinc; essential for growth and development (Krebs and Hambidge, 2007). However, a prospective cohort study in the USA demonstrated low rates of initiation and duration of exclusive breastfeeding, similar to the findings from the presented study, reported formula feeding with the introduction of solids before 4 months of age to be associated with a six fold increased risk of childhood obesity at 3 years of age (Huh *et al.*, 2011). The results of the ongoing follow up of the UPBEAT children at 3-4 years are awaited with interest to determine the long-term influence of early-life feeding on measures of body composition.

Although the protective mechanisms of breastfeeding on childhood obesity are yet to be delineated, it has been suggested that differences in weight gain velocity between infants' breast and formula fed are likely to be attributable to the optimal composition of breast

milk. However, despite some previous reports of breast milk composition in women with GDM; reports suggest a beneficial influence on early weight velocity (Ailhaud *et al.*, 2006; Gunderson, 2007; Nasser *et al.*, 2010).

In contrast, formula milk does not contain bioactive compounds and generally has a higher protein content, both of which may be significant risk factors for later obesity (Patro-Gołąb *et al.*, 2016b). The Childhood Obesity Project provided causal evidence of a high protein formula diet associated with increased growth and weight status at 1 year of age in comparison to a low protein diet. However breastfed infants had significantly reduced weight and length z-scores than formula fed infants at 24 months despite its protein content (Koletzko *et al.*, 2009; Kirchberg *et al.*, 2015).

6.4.4 *Infant appetite and satiety at 6 months of age*

Exclusive breastfed infants were more likely to demonstrate ‘enjoyment of food’ compared to formula or mixed fed infants. This finding was unexpected given the direction of the observed effect sizes on measures of infant anthropometry by mode of early life feeding, at 6 months of age (Llewellyn *et al.*, 2011). The term ‘enjoyment of food’ has consistently been associated with the development of obesity and therefore considered an ‘obesity risk’ characteristics (Llewellyn *et al.*, 2011).

Measures of general appetite assessed by the validated Baby Eating Behaviour Questionnaire were found to be positively associated with measures of infant adiposity, weight z-scores and catch up scores, independent of cord leptin, mode of early feeding, maternal and infant confounding. This finding is similar to previous reports suggesting that the early postnatal environment other than the mode of feeding, may influence general appetite and development of increased adiposity (Reilly *et al.*, 2005; Gorski *et al.*, 2006). These include increased frequency and volume of feeds as well as early introduction of

supplemented milk and beverages which were not assessed within this study. However, the associations with satiety responsiveness with measures of infant adiposity and growth were unexpected as reduced satiety is thought as an obesogenic trait and is associated with increased appetite (Carnell and Wardle, 2009; Syrad *et al.*, 2016). Of interest, a validation study of the Baby Eating Behaviour Questionnaire in an Australian cohort of mother-infant dyads, Mallan MK *et al* demonstrated poor performance of satiety responsiveness as measured by the Baby Eating Behaviour Questionnaire ($\alpha=0.56$) in comparison to the other derived satiety and appetite measures (Mallan *et al.*, 2014) and this may provide an explanation for this unexpected result in this study too.

6.4.5 *Strengths and limitations*

The analysis undertaken here differs from previously published reports as our sample of mothers and their infants were from a prospective cohort, recruited from inner-city populations. As 80% of the population included in this study were in this highest quintile of socioeconomic deprivation comprised entirely of obese women, this study was well placed to assess the mode of early feeding on infant anthropometric outcomes within this high-risk group. Due to the detailed data available, including cord leptin concentrations, potential associations linking early nutrition with later obesity risk are suggested, as well as the assessment of appetite and satiety in infants at 6 months of age. Limitations include the study being undertaken in <30% of the original cohort. However, sensitivity analyses including the use of multiple imputation did not result in differences in the observed relationships. Identification of selection bias may limit translatability of findings to the greater population, however there were no differences in BMI, incidence of gestational diabetes or infant characteristics between those included and excluded within the analyses. The Baby Eating Behaviour Questionnaire is a parent-reported measure and subjected to recall bias. Furthermore, the questionnaire was collected retrospectively, therefore if infants were older than 6 months at the study visit, the majority would have been weaned on to

solids within this cohort. It may also be difficult for mothers to objectively assess two different modes of feeding retrospectively together with potential subjective reporting and bias towards breastfeeding (Llewellyn *et al.*, 2011). Replication of findings using the Baby Eating Behaviour Questionnaire in infants of 3 months of age may provide further insight in appetite and satiety during the predominant milk feeding period. The questionnaire used to assess appetite and satiety did not however distinguish between delivery of breastmilk via breast or bottle which may confound the interpretation of this data as previous studies have suggested that the mode of delivery may influence appetite (Li *et al.*, 2014). The analysis was unable to assess the influence of sweetened beverages or milk supplements given in the early postnatal life.

6.4.6 Conclusion

This study has provided evidence that exclusive breastfeeding may modify early life childhood growth but not adiposity in infants at 6 months of age, born to obese mothers. Future assessment of metabolites within breastmilk, may provide invaluable mechanistic insight associated with this observed protective effect on early life growth. A novel, and potential important association between appetite and adiposity in 6 month infants not previously reported in any study; requires further investigation of the associated mechanisms as suggests that appetite may be a modifiable determinant of adiposity. Ongoing follow-up of this cohort will provide further understanding of the long-term beneficial influence of breastfeeding on later growth and body composition at 3 years of age.

Chapter 7 Discussion

7.1 Main findings of this thesis

This thesis sought to define the associations of the *in-utero* and early life origins of infant adiposity at 6 months of age, born to obese mothers, from a multi-ethnic population associated with high socioeconomic deprivation. The main findings of this thesis are summarised in Figure 7.1.

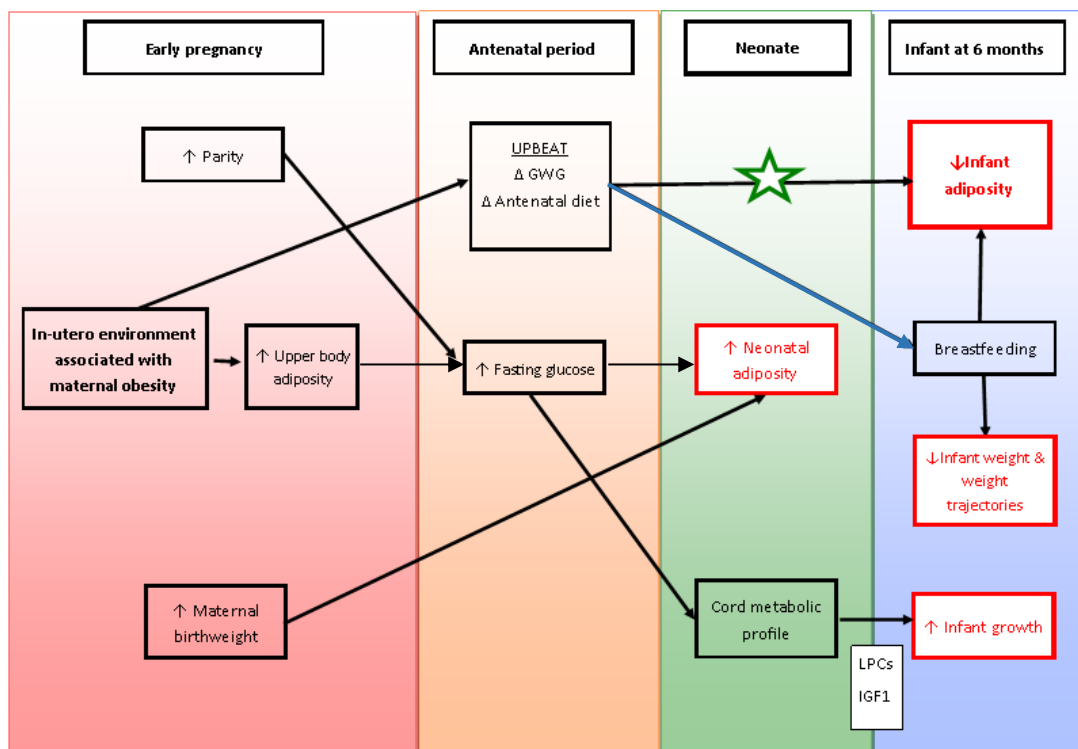


Figure 7.1: Summary of main findings of the analyses incorporated in this thesis.

Observed associations are illustrated in this figure where neonatal adiposity & infant anthropometry are the outcomes of interest (red text). Potential confounders adjusted for within each analysis have not been depicted.

★ Potential causal relationship determined following planned follow-up of the UPBEAT randomised controlled trial. Blue arrow indicates a significant interaction. Abbreviations: GWG- Gestational weight gain; IGF1- Insulin like growth factor; LPCs- lysophosphatidylcholines.

Maternal obesity is associated with greater childhood adiposity and adverse body fat distribution (Patel *et al.*, 2015; Godfrey *et al.*, 2016; Poston *et al.*, 2016). Obesity in early childhood has shown within population studies to be associated with an increased risk of later obesity and cardiometabolic disease (Cunningham *et al.*, 2014; Gaillard *et al.*, 2014b; Perng *et al.*, 2016). As the trajectory for later obesity is thought to be determined at birth and has shown to track through childhood, recognition of effective, targeted interventions within this sensitive window of opportunity are required to curb this increasing epidemic (Cunningham *et al.*, 2014; Giles *et al.*, 2015).

This thesis sought to identify *in-utero* and early life origins associated with neonatal and infant adiposity using data from the UK Pregnancies Better Eating and Physical Activity Trial. Within this cohort of obese women, the work presented has led to the following observations;

- Maternal fasting glucose in late second trimester was found to partially mediate the influence of maternal parity and measures of early pregnancy adiposity on neonatal adiposity. Maternal birthweight was independently associated with neonatal adiposity.
- Higher maternal glycaemia, specifically fasting glucose assessed at late second trimester was linearly associated with cord blood concentrations of lysophosphatidylcholines. Cord blood insulin growth factor I and lysophosphatidylcholines were identified as potential determinants of infant weight at 6 months of age in offspring born to obese women.
- An antenatal lifestyle (dietary and physical activity) intervention in obese pregnant women was found to have the potential to reduce infant adiposity at 6 months of age, and this observation was partially mediated through the changes in maternal gestational weight gain and antenatal diet associated with the intervention.

- In comparison to exclusive breastfeeding, formula and mixed feeding (breast and formula feeding) was associated with increased weight and velocities of weight gain in infants at 6 months of age.

Within this cohort of obese pregnant women, maternal fasting glucose measured in the late 2nd trimester was shown to be an important mediator of early pregnancy characteristics including measures of maternal upper body adiposity and parity. Furthermore, increasing maternal glycaemia was found to be a significant maternal exposure associated with the cord blood metabolic profile. Population studies have consistently demonstrated associations with maternal fasting glucose, neonatal adiposity and subsequent growth (Pettitt *et al.*, 1993; Metzger *et al.*, 2009; Aris *et al.*, 2014; Logan *et al.*, 2016b). Using Mendelian randomisation methodology, the relationship between maternal fasting glucose at 27-28 weeks' and neonatal adiposity was recently proven causal (Tyrrell *et al.*, 2016). The mean BMI from the included studies (n=11 cohort studies included) was 22.8 to 24.8 kg/m², therefore this conclusion may have limited relevance to the greater degree of maternal dysglycaemia observed in obese pregnancies and the subsequent association with neonatal adiposity.

Undertaking mediation analyses as reported in this thesis, facilitated the identification of a critical, intermediary role in the relationship between maternal fasting glucose in the late 2nd trimester (at the time of the OGTT) and neonatal adiposity within a homogeneous obese cohort. There are very few relevant studies in the literature, although Lawlor *et al.*, have demonstrated previously that maternal fasting glucose concentrations at a similar gestation partially mediate the associated risk of Pakistani ethnicity with neonatal fat mass, using cord leptin as a proxy measure, but within a heterogeneous BMI population (Lawlor *et al.*, 2014). Since excessive fetal growth, occurs earlier in pregnancy than at the time of the oral glucose tolerance test (i.e. at the diagnosis of gestational diabetes) (Sovio *et al.*, 2016), it is

also important to understand the earlier pregnancy determinants of neonatal adiposity and this thesis has reported a comprehensive assessment of early maternal characteristics including biochemical variables, the relationships with neonatal adiposity and the associated cord blood metabolic profile. This has provided novel mechanistic insight as well as identification of potential targets for future intervention which may be of benefit in the prevention of neonatal and infant adiposity, in offspring born to obese women.

By undertaking an exploratory analysis, components of the cord metabolic profile, specifically lysophosphatidylcholines and insulin growth factor I were associated with neonatal adiposity and weight, together with measures of infant growth at 6 months of age. Evidence from longitudinal population studies suggest that the trajectory of later obesity may be determined at birth (Tilling *et al.*, 2011; Jones-Smith *et al.*, 2013; Giles *et al.*, 2015). Although studies assessing the cord blood metabolic profile in relation to infant obesity at a later age are limited, one small case-control study has found that metabolite derangements in one-carbon metabolism pathways in cord blood were associated with rapid weight gain within the first 6 months (Isganaitis *et al.*, 2015). That study as well as the report from the UPBEAT cohort presented in this thesis, provide initial evidence that metabolite patterns associated with later disease risk are present at birth and may play a contributory role in the determination of later growth trajectories (Giles *et al.*, 2015; Isganaitis *et al.*, 2015). However, it is not clear if the cord metabolite profile reported here was causally associated with disordered maternal metabolic function in this obese cohort, or whether the fasting/feeding state in before and during delivery could have a potential influence (Isganaitis *et al.*, 2015).

To date, there are no published studies assessing the cord metabolic profile in relation to maternal adverse *in-utero* exposure in obese pregnancies. From the reported UPBEAT study, maternal glycaemia is the strongest contributor to the cord blood metabolic profile in

obese women, including raised lysophosphatidylcholines which, as I have suggested, may act as mediator in the association between maternal glycaemia and offspring adiposity at birth and at 6 months of age. If these findings are proven causal, interventions targeted at improving maternal glycaemic profiles in obese pregnancies may provide a robust approach to reducing offspring adiposity.

In view of the growing incidence of maternal obesity (NCD-RisC, 2016; Poston *et al.*, 2016), several well-powered clinical studies assessing antenatal interventions have been undertaken which have demonstrated small improvements in maternal dietary behaviours and/ or reductions in gestational weight gain. None of these have been associated with a reduction in the incidence of maternal gestational diabetes (Vinter *et al.*, 2011; Walsh *et al.*, 2012; Dodd *et al.*, 2014a; Poston *et al.*, 2015), however as reported in this thesis and recently published (Patel *et al.*, 2017), the intervention in the UPBEAT trial, which changed diet substantially, reduced GWG and maternal adiposity was associated with a reduction in a measure of infant adiposity at 6 months of age. Although a small effect size estimate was observed; this follow-up of a randomised controlled trial, provides for the first time, causal evidence that an intensive behavioural lifestyle intervention has the potential reduce infant adiposity within an obese cohort. Antenatal lifestyle interventions have shown to result in changes in neonatal thigh circumference born to mothers randomised to a low glycaemic diet in pregnancy (Donnelly *et al.*, 2015). However, unlike the UPBEAT studies, previous studies assessing lifestyle interventions in pregnancy have found no difference in offspring anthropometry in early infancy (Vinter *et al.*, 2011; Horan *et al.*, 2016); which may reflect the greater intensity of the UPBEAT intervention leading to improved diet and reduced adiposity in the mother (Poston *et al.*, 2015; Flynn *et al.*, 2016).

By undertaking causal mediation analysis specifically for a randomised controlled trial, changes in total gestational weight gain, energy and saturated fat intake were associated

with the observed difference in infant subscapular skinfold thicknesses. Although the intervention had no effect on the cord blood metabolic profile, in an unpublished report the UPBEAT intervention has been found to lead to a reduction in maternal lipid metabolites, longitudinally assessed at three time points during pregnancy (Lawlor *et al*, manuscript in preparation). Ongoing investigation of the cord epigenome as well as the maternal metabolome may identify causative mechanisms associated with the UPBEAT intervention.

Early life feeding practices in obese populations are considerably different to that of women within a normal BMI range, and this thesis has demonstrated as far as we are aware for the first time, the beneficial role of exclusive breastfeeding in obese women on infant weight and associated velocities in early life; both known predictors of later childhood obesity (Ong *et al.*, 2000; Ong *et al.*, 2006; Ong and Loos, 2006; Kramer *et al.*, 2007). Although small effect estimates were observed, these findings are in line with a previous report undertaken in offspring born to women of heterogeneous BMI (Owen *et al.*, 2005); suggesting that promotion of exclusive breastfeeding in obese women may modify the risk of rapid weight gain in early infancy. Evidence has demonstrated that breastfeeding interventions delivered effectively can result in significant improvements in breastfeeding practices (Rollins *et al.*, 2016). Over the last two decades, the promotion of breastfeeding is no longer considered the sole obligation of the mother but a societal responsibility. The determinants of breastfeeding include sociocultural trends, influence of healthcare systems, employment, family and community environment together with maternal and infant attributes; all potential targets of intervention (Rollins *et al.*, 2016). Methods of intervention include mass advertising, amendments and formulation of policies together with counselling and lactation management at an individual level.

The UPBEAT study was associated with significantly improved maternal dietary behaviours at 6 months postpartum, suggesting that women in the postpartum period are amenable to dietary advice, which may have a beneficial impact within the preconception period for subsequent pregnancies. Systematic reviews and meta-analyses have identified that weight reduction in the postpartum period is achievable in overweight and obese women (van der Pligt *et al.*, 2013) and have demonstrated that breastfeeding duration was associated with maternal weight loss (not reported within this thesis). Interventions which focus on the postpartum period are likely to be more feasible than those within the preconception period, and our group has embarked on an intervention to promote weight loss in women who most at risk of postpartum weight retention (The Swan Study). Targeting women preconceptionally is challenging due to identification and recruitment (Hanson *et al.*, 2016), and would require adoption of a public health intervention approach. Recently the use of 'healthy eating behaviour conversations' provides an approach to modify lifestyle behaviours within a public health setting (Barker *et al.*, 2016); however ongoing work will determine whether this and other similar approaches provide a translatable, effective method to curb the increasing incidence of maternal and subsequent offspring obesity epidemic.

With the exception of Chapter 6, the work reported in this thesis is derived from a prospective observational study, therefore the Bradford Hill criteria should be considered when inferring causality from epidemiological data (Hill, 1965). This criteria was established to increase our confidence that an exposure was causally associated with an outcome providing a criterion was met, which included strength of association, consistency, temporality, biological gradient, plausibility, coherence, experiment and analogy (Hill, 1965). Although the Bradford Hill criteria provides guidelines for inferring causality, each criterion should be applied and interpreted specifically within a data integration framework against new methods of data collection including the use of metabolomics and genomic data. With advances in data collection and the use of

sophisticated epidemiological techniques causal inference is no longer informed by the traditional use of epidemiological techniques but rather a combination of research tools and collaboration amongst scientific disciplines (Fedak *et al.*, 2015).

7.2 Strengths and limitations

This study was conducted within a prospective cohort of obese women, recruited from inner-city, socioeconomically deprived populations. As socioeconomic deprivation has been consistently associated with an increase in all-cause mortality (Smith *et al.*, 1990; Smith *et al.*, 1998), characterising maternal *in-utero* factors associated with offspring adiposity in a population with high socio-economic deprivation, could contribute to improved health of women and children most at risk of sub-optimal health in later life. UPBEAT provides a unique database, including detailed maternal demographic, clinical, familial and environmental characteristics, longitudinal anthropometric, dietary and physical activity assessment; allowing an in-depth examination of the consequences of maternal obesity on offspring adiposity and anthropometry. In addition, follow-up of offspring born to obese women including the collection of anthropometric and dietary data, allows evaluation of the potential role of early life characteristics with subsequent growth together with detailed data on potential confounders. This cohort also provides one of the largest biobanks to date from obese women collected at regular intervals during pregnancy, providing a unique opportunity to address mechanistic relationships between maternal *in-utero* exposures with fetal and infant outcomes. Universal screening of gestational diabetes using the International Association of Diabetes in Pregnancy study group's criteria provided a standardized approach in the classification of an important *in-utero* exposure variable within the reported analyses of this thesis.

As with all longitudinal studies, attrition at follow-up was common for both maternal and offspring data. However, our sample size at follow-up was similar to those of other large

randomised controlled trials (Tanvig *et al.*, 2014; Landon *et al.*, 2015; Horan *et al.*, 2016). In addition, the statistical analysis incorporated within this thesis, acknowledged the introduction of bias associated with missing data and sensitivity analyses did not demonstrate considerable differences in observed effect estimates. The treatment of gestational diabetes has been shown to alter the maternal metabolic environment especially within the third trimester and this may modify offspring adiposity deposition (Stumvoll *et al.*, 1995; Ratner *et al.*, 2008; Rowan *et al.*, 2011). This study did not have a standardised approach for the treatment of gestational diabetes in participants which may have resulted in differences within pharmacological intervention between the centres. The study also lacked detailed data on initiation and duration of different modalities of treatment for gestational diabetes. Within epidemiological research, assessing the influence of different treatment modalities on outcomes is challenging, as the modalities of treatment (including capillary blood testing, dietary advice, metformin and/or insulin) overlap considerably. To consider any potential effect, analyses were adjusted for the incidence of gestational diabetes, a proxy variable for the initiation of treatment of gestational diabetes. Furthermore, detailed sensitivity analyses were undertaken excluding participants diagnosed with gestational diabetes, to assess for potential modification in observed effect estimates. Infant and maternal dietary data at 6 months postpartum, were collected with the use of validated questionnaires (Bingham *et al.*, 2001; Robinson *et al.*, 2007; Briley *et al.*, 2014), which may be subjected to recall bias. However, at the time of undertaking this study, and to date the use of questionnaires is the accepted and established method of collecting dietary data within a population cohort.

7.3 Recommended future work

Ongoing follow-up of the offspring at 3 years of age, will provide valuable data to assess whether the UPBEAT intervention led to long-term, sustained improvements in offspring adiposity. Skinfold thicknesses and circumferences are being collected as well as valuable

bioelectrical impedance data. Furthermore, combining the cord blood metabolomics data, with longitudinal anthropometric data, will provide further understanding of the mechanistic pathways implicated with the development of childhood adiposity. As the cord metabolomics analyses, identified no effect of the UPBEAT intervention; cord blood epigenetic analysis currently being undertaken, may identify potential mediators associated with the observed change in infant central fat mass at 6 months of age.

Maternal second trimester fasting glycaemic profile was shown to play a critical role in the development of neonatal adiposity and cord blood metabolic profile. Work from this thesis could be extended by undertaking continuous glucose monitoring in obese pregnant women. This would allow the characterisation of subtle variations in maternal antenatal glucose in relation to offspring adiposity throughout the life course. Furthermore, the provision of comprehensive glucose data, would allow the characterisation and evaluation of different modalities of gestational diabetes treatment with outcomes in early infancy.

As maternal obesity places an increased burden on healthcare resources, universal screening and subsequent treatment for all obese women does not provide a cost-effective solution for the prevention of adverse offspring outcomes (Farrar *et al.*, 2016b). Early detection and treatment of gestational diabetes by metformin in women most at risk, using for example, the GDM risk assessment tool recently developed in the UPBEAT cohort (White *et al.*, 2016), in combination with a dietary and physical activity intervention, may provide a cost-effective solution to modify maternal fasting glycaemic profiles, gestational weight gain and lifestyle behaviours to the postpartum period (Poston *et al.*, 2013; Poston *et al.*, 2015; Flynn *et al.*, 2016; White *et al.*, 2016).

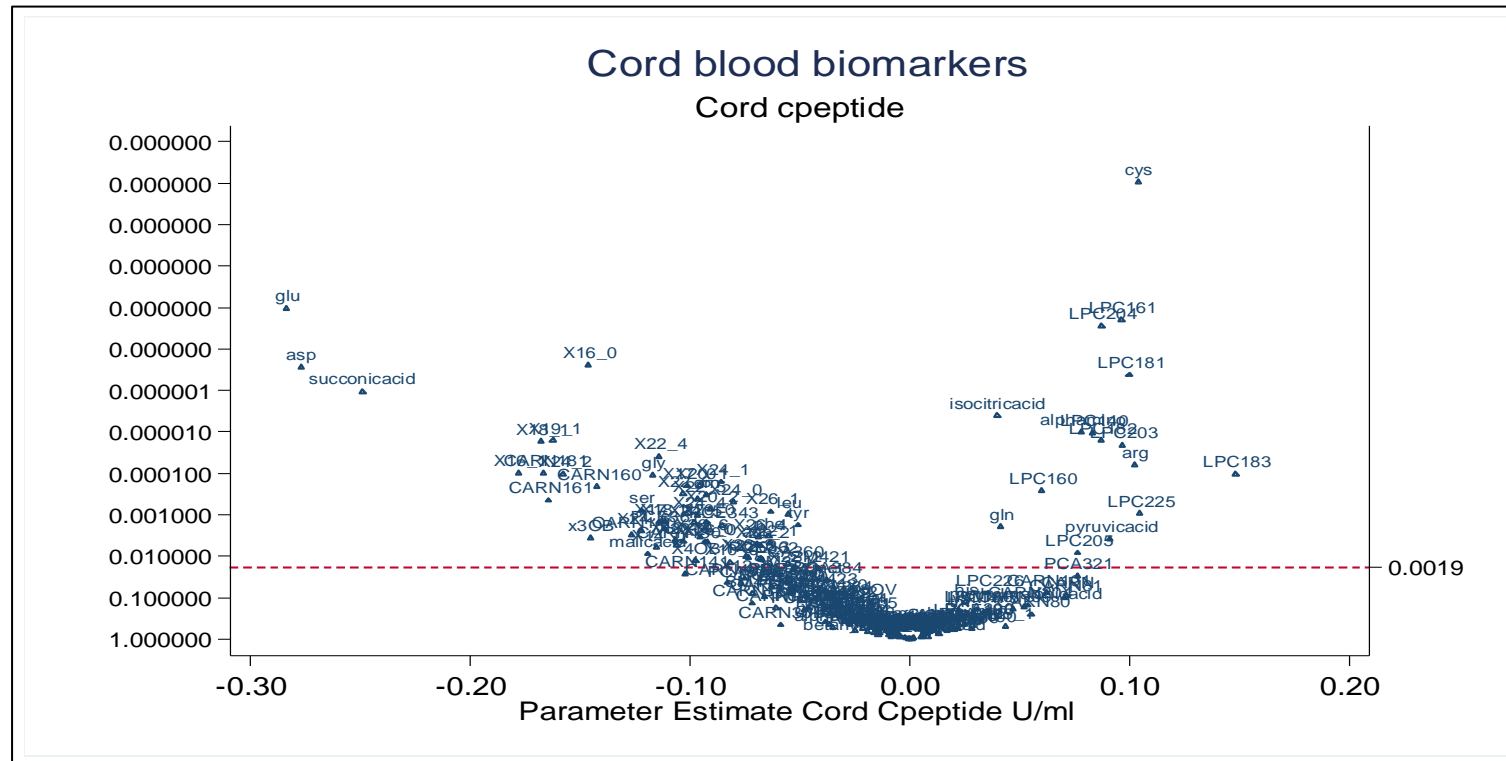
7.4 Conclusion

Using data from the UPBEAT cohort, this thesis has demonstrated the following key observations.

- In offspring born to obese pregnant women, maternal fasting glucose in the late second trimester partially mediates the influence of maternal parity and measures of early pregnancy adiposity on neonatal adiposity.
- Cord blood insulin growth factor I and lysophosphatidylcholines are potential determinants of infant weight at 6 months of age in offspring born to obese women.
- An antenatal lifestyle (dietary and physical activity) intervention in obese pregnant women has the potential to reduce infant adiposity at 6 months of age, partially mediated through changes in maternal gestational weight gain and antenatal diet associated with the intervention. This finding suggests a causal association between improvements in obese maternal diet and physical activity is associated with a reduction in infant adiposity.
- In comparison to exclusive breastfeeding, formula and mixed feeding (breast and formula feeding) was associated with increased weight and velocities of weight gain in infants of obese mothers at 6 months of age.

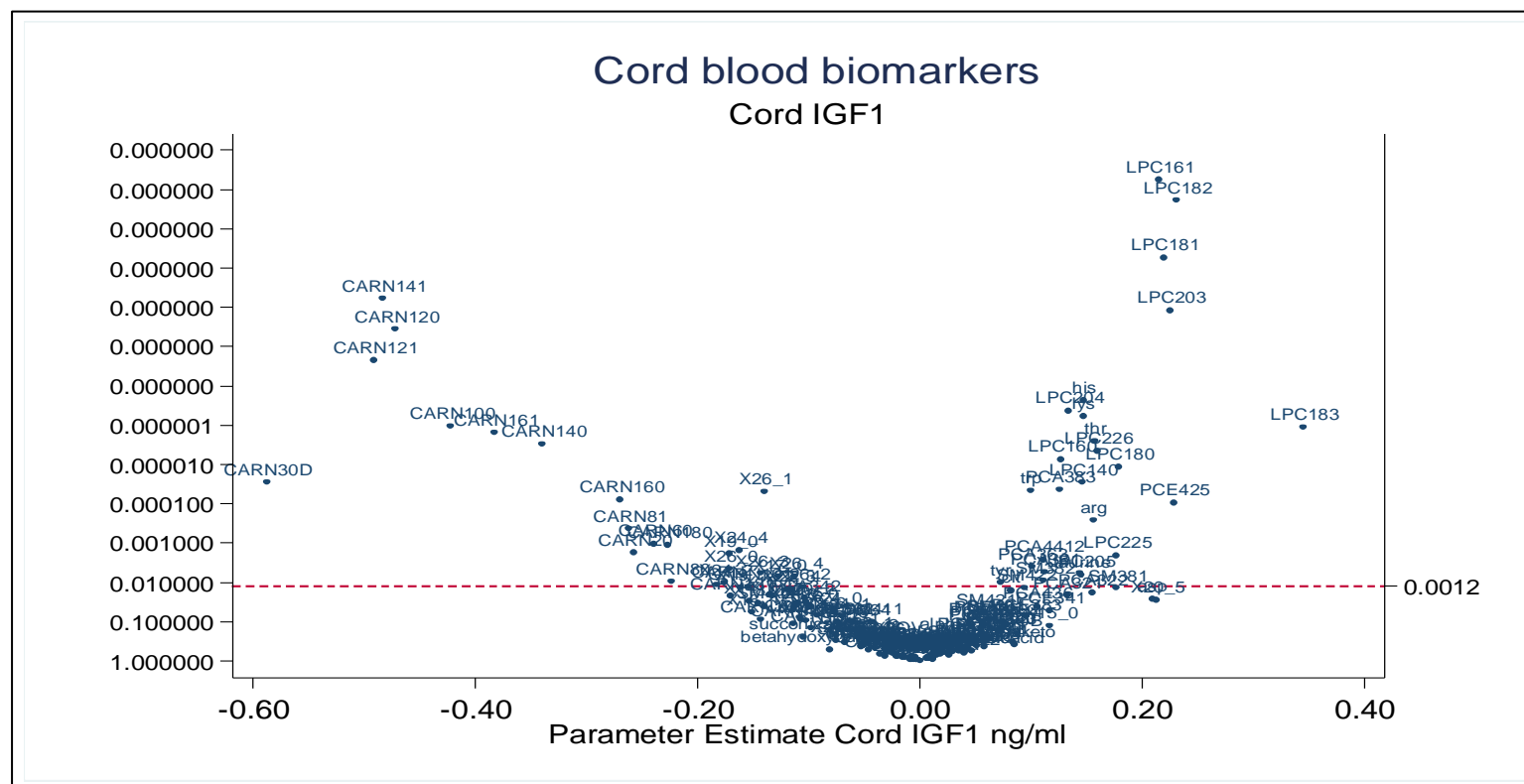
If these findings are proven casual, this study will have provided evidence towards the development of interventions to improve maternal fasting glucose, which in combination with a lifestyle intervention in the antenatal period and promotion of breastfeeding in the postnatal period, has the potential to curb the increasing incidence of infant obesity.

Appendix



Appendix Figure 1: Association of cord blood metabolomics profile with cord C-peptide in obese pregnant mothers; data from the UPBEAT study (n=343).

Parameter estimates are graphically represented for each biochemical variable in relation to cord blood C-peptide allowing correction for a false discovery rate (Benjamin & Hochberg procedure) (Benjamini and Hochberg, 1995). Adjustment made for randomisation to the UPBEAT intervention, offspring sex and gestation at delivery. Statistical significance $p \leq 0.0019$. Abbreviations; CARN-Carnitines; HDL-High density lipoprotein; IGF I- Insulin growth factor I; IGF II- Insulin growth factor II; IL6- Interleukin 6; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines; SM- sphingomyelins. TNFalpha- Tumor necrosis factor; X30B- x3methyl2oxobutanoicacid; X30V-- x4methyl2oxovalericacid. NEFAs are described using the nomenclature CX:Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.



Appendix Figure 2: Association of cord blood metabolomics profile with cord IGF-1 in obese pregnant mothers; data from the UPBEAT study (n=343).

Parameter estimates are graphically represented for each biochemical variable in relation to cord blood IGF-1 allowing correction for a false discovery rate (Benjamin & Hochberg procedure) (Benjamini and Hochberg, 1995). Adjustment made for randomisation to the UPBEAT intervention, offspring sex and gestation at delivery. Statistical significance $p \leq 0.0019$. Abbreviations; CARN-Carnitines; HDL-High density lipoprotein; IGF1- Insulin growth factor I; IGF II- Insulin growth factor II; IL6- Interleukin 6; LPC- lysophosphatidylcholines; LPCE- lysophosphatidylethanolamine; PC-phosphatidylcholines; PCA- diacylphosphatidylcholines; PCE- acylalkylphosphatidylcholines; SM- sphingomyelins. TNFalpha- Tumor necrosis factor; X30B- x3methyl2oxobutanoicacid; X30V- x4methyl2oxovalericacid. NEFAs are described using the nomenclature CX:Y, where X is the length of the carbon chain, Y is the number of double bonds, OH in the formula means the molecule contains a hydroxyl-group.

Thesis publications

Chapter 1: Introduction

1. **PATEL N**, Poston L. Maternal Obesity and Gestational Weight Gain as Determinants of Long-Term Health. Parental Obesity: Intergenerational Programming and Consequences 2016; doi: 978-1-4939-6384.
2. **PATEL N**, Pasupathy D, Poston L. Determining the Consequences of Maternal Obesity on Offspring Health. Experimental Physiology 2015; doi: 10.1113/EP085132.
3. Poston L, **PATEL N**. Dietary Recommendations for Obese Pregnant Women: Current Questions and Controversies. Acta Obstetrica et Gynecologica Scandinavica 2014;11; 1081-84

Chapter 2: Methods

1. Flynn AF, Goff L, Seed PT, **PATEL N** et al. Dietary patterns in obese pregnant women; influence of a behavioural intervention of diet and physical activity in the UPBEAT randomized controlled trial. International Journal of Behavioural Nutrition and Physical Activity. 2016;13:124-128.
2. Poston L, .Bell R, Croker H, Flynn AC, Godfrey KM, Goff L, Hayes L, Khazaezadeh N, Nelson SM, Oteng-Ntim E, Pasupathy D, **PATEL N**, Robson SC , Sandall J, Sanders TAB, Seed PT, Sattar N, Wardle J, Whitworth MK, Briley AL. A Multicentre Randomised Controlled Trial of a Behavioural Intervention in Obese Pregnant Women; the UPBEAT Study. Lancet Diabetes & Endocrinology 2015;3:767-777.
3. Briley AL, Barr S, Badger S, Bell R, Croker H, Godfrey KM, Holmes B, Kinnunen TI, Nelson SM, Oteng- Ntim E, **PATEL N**, Robson SC, Sandall J, Sanders T, Sattar N, Seed PT, Wardle J, Poston L. A Complex Intervention to Improve Pregnancy Outcome in Obese Women; the UPBEAT Randomised Controlled Trial. BMC Pregnancy and Childbirth 2014; 14:74-87.

Chapter 3: Relation of clinical and metabolic characteristics to neonatal adiposity among obese pregnant women.

1. **PATEL N**, Pasupathy D, Bell R, Briley AB, Godfrey KM, Lawlor DA, Nelson SM, Oteng-Ntim E, Robson SC, Sattar N, Seed PT, Singh C, Wardle J, Welsh P, Poston L on behalf of the UPBEAT Consortium. Relation Of Clinical And Metabolic Characteristics To Neonatal Adiposity Among Obese Pregnant Women. International Journal of Obesity; Manuscript drafted.

Chapter 4: Cord blood metabolic profiles in obese pregnant women; insights into offspring growth and body composition

1. **PATEL N**, Hellmuth C, Godfrey KM, Briley A, Welsh P, Pasupathy D, Koletzko B, Poston L on behalf of the UPBEAT Consortium. Cord blood metabolic profile in obese pregnant women; insights into offspring growth and body composition. *Journal of Clinical Endocrinology and Metabolism*; Manuscript submitted.

Chapter 5: Infant adiposity following a randomised controlled trial of a behavioural intervention in obese pregnancy.

1. **PATEL N**, Godfrey KM, Pasupathy D, Levin J, Flynn AF, Hayes L, Briley AO, Bell R, Lawlor DA, Oteng-Ntim E, Nelson S, Robson S, Sattar N, Singh C, Wardle J, White S, Seed PT, Poston L on behalf of the UPBEAT Consortium. Infant adiposity following a randomised controlled trial of a behavioural intervention in obese pregnancy. *International Journal of Obesity*, Accepted 2017.

Chapter 6: Role of early feeding on infant anthropometry

1. Briley A & **PATEL N**, Seed PT, Poston L on behalf of the UPBEAT Consortium. Role of early feeding, appetite and satiety on infant and maternal anthropometry. Manuscript drafted.

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